EPA/635/R-11/006F
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TOXICOLOGICAL REVIEW

OF

TETRAHYDROFURAN

(CAS No. 109-99-9)

In Support of Summary Information on the Integrated Risk Information System (IRIS)

February 2012

U.S. Environmental Protection Agency
Washington, DC

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ABBREVIATIONS AND ACRONYMS

ABT	1-aminobenzotriazole
ACGIH	American Conference of Governmental Industrial Hygienists
AIC	Akaike Information Criterion
ALT	alanine aminotransferase
AST	aspartate aminotransferase
ATH	atypical tubule hyperplasia
ATPase	adenosine triphosphatase
AUC	area under the curve
BASF	Badische Anilin- und Sodafabrik
BMC	benchmark concentration
BMCL	95% lower bound on the BMC
BMD	benchmark dose
BMDL	95% lower bound on the BMD
BMDS	BMD software
BMR	benchmark response
BPE	benzo[a]pyrene-trans-7,8-dihydrodiol-9,10-epoxide
BrdU	5-bromo-2-deoxyuridine
BW	body weight
CASRN	Chemical Abstracts Service Registry Number
cDNA	complementary DNA
C_{max}	maximum plasma concentration following administration of a chemical
CNS	central nervous system
CO_2	carbon dioxide
CPN	chronic progressive nephropathy
CYP450	cytochrome P450
dUTP	deoxyuridine triphosphate
EEG	electroencephalogram
EPA	U.S. Environmental Protection Agency
EROD	ethoxyresorufin-O-deethylase
FOB	functional observational battery
GABA	γ-aminobutyric acid
GBL	γ-butyrolactone
GGT	γ-glutamyl transferase
GHB	γ-hydroxybutyric acid
GI	gastrointestinal
GJIC	gap junctional intercellular communication
HEC	human equivalent concentration
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
LA	labeled area
LC	labeled cell
LC_{50}	median lethal concentration

LD_{50}	median lethal dose
LI	labeling index
LOAEL	lowest-observed-adverse-effect level
LOD	limit of detection
LOEL	lowest-observed-effect level
MI	mitotic index
MOA	mode of action
$NADP^+$	nicotinamide adenine dinucleotide phosphate
NADPH	the reduced form of $NADP^+$
NCI	National Cancer Institute
NIOSH	National Institute for Occupational Safety and Health
NOAEL	no-observed-adverse-effect level
NPH	nitrophenol hydroxylase
NRC	National Research Council
NSF	National Sanitation Foundation
NTP	National Toxicology Program
PBPK	physiologically based pharmacokinetic
PCNA	proliferating cell nuclear antigen
PI_{50}	50% reduction of cell protein content
PND	postnatal day
POD	point of departure
PON	paraoxonase
PROD	pentoxyresorufin-O-depentylase
PWG	Pathology Working Group
RBC	red blood cell
RfC	reference concentration
RfD	reference dose
RGDR	regional gas dose ratio
SD	standard deviation
SSA	succinic semialdehyde
$T_{1/2}$	half-life
THF	tetrahydrofuran
T_{max}	the time after administration of a chemical when the maximum plasma concentration is reached; when the rate of absorption equals the rate of elimination
TUNEL	terminal deoxynucleotidyl dUTP nick-end-labeling staining
TWA	time-weighted average
UF	uncertainty factor
VOC	volatile organic compound

FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard and dose-response assessment in IRIS pertaining to chronic exposure to tetrahydrofuran. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of tetrahydrofuran.

The intent of Section 6, *Major Conclusions in the Characterization of Hazard and Dose Response*, is to present the major conclusions reached in the derivation of the reference dose, reference concentration and cancer assessment, where applicable, and to characterize the overall confidence in the quantitative and qualitative aspects of hazard and dose response by addressing the quality of data and related uncertainties. The discussion is intended to convey the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's IRIS Hotline at (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address).

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1. INTRODUCTION

This document presents background information and justification for the Integrated Risk Information System (IRIS) Summary of the hazard and dose-response assessment of tetrahydrofuran (THF). IRIS Summaries may include oral reference dose (RfD) and inhalation reference concentration (RfC) values for chronic and other exposure durations, and a carcinogenicity assessment.

The RfD and RfC, if derived, provide quantitative information for use in risk assessments for health effects known or assumed to be produced through a nonlinear (presumed threshold) mode of action. The RfD (expressed in units of mg/kg-day) is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The inhalation RfC (expressed in units of mg/m^3) is analogous to the oral RfD, but provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory or systemic effects). Reference values are generally derived for chronic exposures (up to a lifetime), but may also be derived for acute (≤24 hours), short-term (>24 hours up to 30 days), and subchronic (>30 days up to 10% of lifetime) exposure durations, all of which are derived based on an assumption of continuous exposure throughout the duration specified. Unless specified otherwise, the RfD and RfC are derived for chronic exposure duration.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral and inhalation exposure may be derived. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates may be derived from the application of a low-dose extrapolation procedure. If derived, the oral slope factor is a plausible upper bound on the estimate of risk per mg/kg-day of oral exposure. Similarly, an inhalation unit risk is a plausible upper bound on the estimate of risk per $\mu g/m^3$ air breathed.

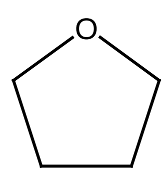
Development of these hazard identification and dose-response assessments for tetrahydrofuran has followed the general guidelines for risk assessment as set forth by the National Research Council (NRC, 1983). EPA Guidelines and Risk Assessment Forum technical panel reports that may have been used in the development of this assessment include the following: *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986a), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986b), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), *Guidelines* *for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991a), *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994a), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998), *Science Policy Council Handbook*: *Risk Characterization* (U.S. EPA, 2000a), *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000b), *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 2000c), *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002), *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 2006a), and *A Framework for Assessing Health Risks of Environmental Exposures to Children* (U.S. EPA, 2006b).

The literature search strategy employed for tetrahydrofuran was based on the chemical name, Chemical Abstracts Service Registry Number (CASRN), and multiple common synonyms. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document. Primary, peer-reviewed literature identified through December 2010 was included where that literature was determined to be critical to the assessment. The relevant literature included publications on tetrahydrofuran that were identified through Toxicology Literature Online (TOXLINE), PubMed, the Toxic Substance Control Act Test Submission Database (TSCATS), the Registry of Toxic Effects of Chemical Substances (RTECS), the Chemical Carcinogenesis Research Information System (CCRIS), the Developmental and Reproductive Toxicology/Environmental Teratology Information Center (DART/ETIC), the Hazardous Substances Data Bank (HSDB), the Genetic Toxicology Data Bank (GENE-TOX), Chemical abstracts, and Current Contents. Other peer-reviewed information, including health assessments developed by other organizations, review articles, and independent analyses of the health effects data were retrieved and may be included in the assessment where appropriate. It should be noted that references have been added to the Toxicological Review after the external peer review in response to public comments and for the sake of completeness. The addition of these references has not changed the overall qualitative and quantitative conclusions. See Section 7 for a list of the references added after peer review.

2. CHEMICAL AND PHYSICAL INFORMATION

Tetrahydrofuran (THF) is a synthesized organic compound that is not found in the natural environment (ACGIH, 2001). It is a colorless, volatile liquid with an ethereal or acetone-like smell and is miscible in water and most organic solvents. Table 2-1 summarizes the physical and chemical properties of THF. THF is highly flammable and upon thermal decomposition may form carbon monoxide and carbon dioxide. Under certain conditions, such as prolonged storage in contact with air, THF can decompose into explosive peroxides.

Table 2-1. Chemical and physical properties of THF

CAS Registry Number	109-99-9	Verschueren (2001)
Synonym(s)	THF; diethyleneoxide; tetramethyleneoxide; 1,4 -epoxy butane; furanidine; oxacyclopentane	Verschueren (2001)
Melting point, °C	-108.5	Verschueren (2001)
Boiling point, °C	65/66	Verschueren (2001)
Vapor pressure, atm at 20°C	0.173	Verschueren (2001)
Density, at 20°C relative to the density of H_2O at 4°C	0.89	Verschueren (2001)
Flashpoint (closed cup)	-1 to -21.5°C	BASF (1993)
Water solubility	Miscible	NIOSH (1997)
Log K_{ow}	0.46	SRC (2001)
Odor threshold	2–7.4 ppm 60–150 mg/m^3	ACGIH (2001); RIVM (2001)
Molecular weight	72.10	Verschueren (2001)
Conversion factors	1 ppm = 2.95 mg/m^3	NIOSH (1997)
Empirical formula	C_4H_8O	Verschueren (2001)
Chemical structure		Verschueren (2001)

THF is used as a solvent for polyvinyl chlorides, vinylidene chloride polymers, and natural and synthetic resins (particularly vinyls), and in topcoating solutions, polymer coatings, cellophane, protective coatings, adhesives, magnetic strips, and printing inks. It is also used for Grignard and metal hydride reactions. THF is used as an intermediate in chemical synthesis. For example, it is used in the preparation of chemicals, including adipic acid, butadiene, acrylic acid, butyrolactone, succinic acid, 1,4-butanediol diacetate, motor fuels, vitamins, hormones, pharmaceuticals, synthetic perfumes, organometallic compounds, and insecticides. It is also used in the manufacture of polytetramethylene ether glycol, polyurethane elastomers, and elastic polymers. THF can be used in the fabrication of materials for food packaging, transport, and storage. When THF is used in food processing, it can be an indirect food additive (National Toxicology Program [NTP], 1998).

Potential exposures to humans result from anthropogenic sources, primarily from occupational exposures related to THF's use as a solvent for resins, adhesives, printers' ink, and coatings. Exposure to THF is primarily through inhalation or dermal absorption in the workplace. Nonoccupational exposure is uncommon, but may occur via inhalation and oral routes from contamination of the environment (air and water) (NTP, 1998).

3. TOXICOKINETICS

3.1. ABSORPTION

3.1.1. Gastrointestinal Absorption

No information on THF absorption from the human gastrointestinal (GI) tract is available. However, blood and tissue concentration data from a toxicokinetic study in rats and mice conducted by DuPont Haskell Laboratory (1998) have demonstrated that THF is readily absorbed from the GI tract. In this study, single gavage doses of approximately 50 or 500 mg/kg [^{14}C]-THF dissolved in water were administered to male and female F344 rats and B6C3F$_1$ mice, and the level of THF-associated radioactivity in plasma was monitored for up to 168 hours. The mean values of selected toxicokinetic parameters for plasma identified in this study are presented in Table 3-1.

In both rats and mice, radioactivity appeared in the plasma soon after the THF treatment, demonstrating the rapid absorption of THF from the GI tract. In rats, detectable levels of radioactivity were present in the plasma as early as 15 minutes after dosing (the earliest time point measured). Maximum plasma concentrations were reached after approximately 4 hours in the low-dose rats and after 4–8 hours in the high-dose rats. In the low-dose group, the plasma concentration reached a maximum (C_{max}) of 19.8 μg THF equivalents/g in males at 4 hours and 13.8 μg THF equivalents/g in females at 3 hours. In the high-dose group, the C_{max} was 71.6 μg THF equivalents/g plasma in males at 8.0 hours and 89.2 μg THF equivalents/g plasma in females at 3.2 hours. The T_{max} (the time after administration of a chemical when the maximum plasma concentration is reached; when the rate of absorption equals the rate of elimination) in females was highly variable. Maximum plasma concentrations were not proportional to the administered dose, since C_{max} values differed by approximately fourfold for males and sevenfold for females between dose groups, while the administered dose differed by 10-fold.

A similar evaluation of the plasma area under the curve (AUC) data revealed the same pattern of nonproportionality with dose. This phenomenon could reflect the saturability of absorption processes at high doses. Also, independent of absorption, dose-dependent changes in first-pass metabolism could possibly explain this result. Since GI tract absorption rates have not been measured directly, the data are not adequate to attribute the nonlinearity in maximum plasma concentrations or AUCs to absorption kinetics. As the values of many of the kinetic parameters are highly variable (Table 3-1), the study authors (DuPont Haskell Laboratory, 1998) indicated that there were no gender differences for any of the kinetic parameters in the rat (statistical significance not reported by the study authors).

Table 3-1. Toxicokinetic parameters in rat and mouse plasma following a single gavage administration of [^{14}C]-THF

	Dose: 50 mg/kg		Dose: 500 mg/kg	
	Male	**Female**	**Male**	**Female**
Rat				
Actual dose (mg/kg)	40.3	45.9	428.7	478.3
T_{max} (hrs)	4.0	3.0	8.0	3.2
C_{max} (µg equivalents/g)	19.8	13.8	71.6	89.2
$T_{1/2}$ (hrs)	52.1	50.5	48.0	59.0
AUC (µg•hr/g)	535.8	319.6	2,825.5	1,998.0
Clearance (g/hr•kg)	75.2	143.6	151.7	239.4
Mouse				
Actual dose (mg/kg)	44.3	38.0	490.3	495.9
T_{max} (hrs)	0.5	0.4	0.8	1.0
C_{max} (µg equivalents/g)	27.7	19.4	149.4	106.0
$T_{1/2}$ (hrs)	56.9	51.4	57.3	98.5
AUC (µg•hr/g)	207.4	157.3	3,237.9	1,904.4
Clearance (g/hr•kg)	213.6	241.6	151.4	260.4

$T_{1/2}$ = half-life.

Source: Adapted from data in DuPont Haskell Laboratory (1998); data are expressed as mean values.

Similar to the observations in the rat, THF-associated radioactivity appeared rapidly in mouse plasma after gavage dosing. Fifteen minutes following the 50 mg/kg treatment, a mean value of 17.4 µg THF equivalents/g plasma was observed in females while no radiolabel was detected in males at this sampling time. Following the 500 mg/kg treatment, the mean values at 15 minutes were 84.8 and 56.8 µg THF equivalents/g plasma for males and females, respectively. In the 50 mg/kg dose group, plasma radioactivity reached the C_{max} of 27.7 and 19.4 µg THF equivalents/g at approximately 30 minutes after dosing in males and females, respectively. In the 500 mg/kg group, the plasma radioactivity reached C_{max} values of 149.4 and 106.0 µg THF equivalents/g at approximately 1 hour after dosing in males and females, respectively. No differences were observed for the mouse T_{max} values between either sex (statistical significance not reported by the study authors). The mouse T_{max} values were shorter than for the parallel dose-groups in rats, suggesting that the absorption of THF is more rapid in mice than in rats. As was observed in rats, the C_{max} values in mice were not proportional to the administered dose. However, evaluation of the plasma AUC data for mice suggested that the total absorbed dose was more than proportional to the administered doses; the AUC was 12-fold higher at the high dose in females and 16-fold higher at the high dose in males as compared to the AUC in the corresponding low-dose groups. The lack of proportionality of the C_{max} and AUC is consistent with an effect of dose on absorption rate. However, effects of other kinetic parameters such as metabolism could explain these observations, and therefore, the apparent nonlinearity in plasma kinetics cannot be attributed only to absorption.

The oral bioavailability of THF has not been assessed directly. However, measurement of THF-associated radioactivity in the excreta of the rats and mice in the toxicokinetics study by DuPont Haskell Laboratory (1998) suggests that most (if not all) of orally administered doses of THF can be absorbed. In rats and mice, the total radioactivity recovered in urine, feces, expired air (carbon dioxide [CO_2] or volatile organics), tissues, cage wash, and residual feed was measured over a period of 168 hours after gavage dosing (Table 3-2). The total recovery of radioactivity (i.e., mass balance) was low in both dose groups of rats and the high-dose group of mice, which was attributed by the study authors to saturation in the CO_2 capture system at early time points after dosing and limited performance of the solvent used to capture volatile organics. However, changes in the apparatus for collection of CO_2 and volatile organics employed for the low-dose mice yielded much better recovery of the administered radioactivity. Analysis of data from the low-dose mice shows that little THF remains unabsorbed from the GI tract, since recovery of radioactivity in the feces did not account for more than 1.4% of the administered dose. The amount of THF-associated radioactivity recovered in the feces in these treatment groups was similar to the low-dose mice, suggesting that THF is nearly completely absorbed following oral dosing of up to 500 mg/kg in rats and mice.

Table 3-2. Overall percent recovery of radioactivity at 168 hours following gavage administration of [^{14}C]-THF

	Dose: 50 mg/kg				Dose: 500 mg/kg			
	Rat		Mouse		Rat		Mouse	
Sample[a]	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Urine	4.4	3.5	2.7	5.3	2.2	2.2	3.8	3.6
Feces	1.1	1.0	1.4	0.9	1.0	0.4	1.3	0.8
CO_2	47.8	47.5	58.2	74.6	21.9	18.8	51.1	36.2
Volatile organics	<LOD[b]	<LOD	17.8	24.5	<LOD	<LOD	0.3	0.2
Tissues	14.1	9.3	3.8	2.0	7.9	4.1	4.4	0.7
Cage wash and residual feed	<LOD	<LOD	1.3	1.2	<LOD	<LOD	1.1	1.9
Total	67.5	61.3	85.2	108.5	33.0	25.5	61.9	43.3

[a]This table contains data from only those individual rats that had all listed samples collected.
[b]LOD = Limit of detection.

Source: DuPont Haskell Laboratory (1998).

3.1.2. Respiratory Tract Absorption

The results from several human studies show that THF is readily absorbed from the respiratory tract. A study of workers in a videotape manufacturing plant (Ong et al., 1991) suggested that THF is absorbed by the inhalation route. In a group of 58 workers, full shift personal sampling was conducted to estimate breathing zone concentrations of THF. THF concentrations in the blood, exhaled air, and urine of the workers were determined at the end of the final work shift of the workweek. Time-weighted average exposures ranged from 0.2 to 143.0 ppm (0.59–422 mg/m^3). The measured air concentrations correlated best with urinary THF levels (0.88), followed by blood (0.68), and exhaled air (0.61). A limitation of the study was the inability to estimate the rate of THF absorption from the respiratory tract since the overall contribution of dermal exposure (described as extensive for some workers) and the systemic THF levels were not determined. It was also unclear whether dermal exposure might correlate with THF levels in breathing zone air. Another study of THF workers (Ong et al., 1991) reported that the degree of THF absorption from the respiratory tract is 70% under heavy workloads and 60% during normal breathing.

Kageyama (1988) investigated the toxicokinetics of THF in volunteers exposed by the inhalation route. In the first experiment, subjects (1–20 per group) were exposed for 6 minutes to THF concentrations of 108–395 ppm, and exhaled air was sampled. The authors calculated the THF uptake ratio based on the concentrations of THF in the inhaled air divided by the concentration of THF in the exhaled air. The average uptake ratio was 64.8% for males and 72.7% for females during normal breathing and 78.4% for males and 81.3% for females during deep breathing. No consistent concentration-related effects on uptake were apparent. These results suggested that as much as 81.3% of the THF was absorbed or retained in the lung under acute exposure conditions. In a second experiment, five male subjects were exposed for 3 hours to mean concentrations of 56 ppm THF, followed by a 1-hour recovery period and then a second 3-hour exposure. Exhaled air was monitored throughout the first 3-hour exposure period. The percentage of THF in expired air relative to inhaled air was reported as 40% during normal breathing and 27% during deep breathing. These results correspond to uptake ratios of 60 and 73%, respectively. The same results were observed for five male subjects exposed for a single 3-hour exposure period to a mean THF concentration of 193 ppm THF (experiment 3). The authors also exposed five male volunteers to approximately 200 ppm (207 ppm for first exposure and 178 ppm for second exposure) THF for sequential 3-hour exposure periods with a 1-hour recovery period in between (experiment 4). Blood samples were collected for several of the exposure protocols (experiments 2, 3, and 4). THF kinetics in blood were highly variable among individuals. However, the appearance of THF in the blood demonstrates the systemic absorption of THF from the lungs in exposed humans.

Wagner (1974) also reported on the respiratory tract absorption of THF in four volunteers. The volunteers were exposed to 100 ppm THF for 20 minutes. The absorption rate of THF was reported to be 60%. The author suggested that the reported absorption rate represented 80% of the steady-state absorption rate normally reached over a period of several hours. This value is similar to reports in other human volunteer studies (Teramoto et al., 1989; Kageyama, 1988).

Tissue distribution studies in animals also provide evidence for absorption of THF through the respiratory tract, since measurable levels of THF were found in a variety of tissues in rats exposed through the inhalation route (Elovaara et al., 1984; Kawata and Ito, 1984).

3.1.3. Dermal Absorption

Limited information is available on the dermal absorption of THF in either humans or animals. Systemic toxicity observed in acute dermal toxicity studies (Stasenkova and Kochetkova, 1963) showed that THF can be absorbed through the skin. Brooke et al. (1998) demonstrated that uptake of vapor of industrial solvents across the skin can also occur in humans, but the degree of dermal uptake appears to be negligible (compared to inhalation). Under the conditions of the study in which four volunteers, two with and two without masks, were exposed to 150 ppm THF vapor for 4 hours, dermal uptake of THF vapor (in volunteers with masks) was found to contribute around 1–2% of the body burden received following whole-body (including inhalation) exposure (in volunteers without masks).

3.2. DISTRIBUTION

No tissue distribution studies have been conducted for humans exposed to THF by any route of exposure. However, Ong et al. (1991) reported that occupational exposures (potentially inhalation and dermal) to THF resulted in measurable blood and urine THF levels. Kageyama (1988) and Droz et al. (1999) reported measurable blood concentrations of THF in volunteers exposed by the inhalation route. These results demonstrate the potential for wide tissue distribution of THF.

Tissue distribution of THF has been studied comprehensively in rats and mice following oral dosing (DuPont Haskell Laboratory, 1998). Single gavage doses of [^{14}C]-THF at target concentrations of 50 or 500 mg/kg were administered to male and female F344 rats or B6C3F$_1$ mice, and radioactive residues were measured in the plasma, red blood cells (RBCs), skin, whole blood, bone marrow, brain, fat, heart, lungs, spleen, liver, kidney, GI tract and GI tract contents, ovaries, testes, adrenals, plasma, uterus, muscle, bone, and carcass.

For rats, plasma and RBCs were collected at multiple time points, and, at 168 hours after dosing, the animals were sacrificed and tissues were harvested for analysis of THF-associated radioactivity. The presence of radioactivity in plasma demonstrates that THF or its metabolites are available for systemic distribution. Comparison of kinetic data for plasma and RBCs provides information on partitioning of THF (or its metabolites) in the blood compartment. The C_{max} values for plasma were consistently higher than C_{max} values for RBCs, ranging from 2.7- to 4.8-fold among both dose groups in males and females. When the AUC data are compared for plasma versus RBCs, the opposite relationship was observed (i.e., AUC values were higher in RBCs than in plasma), consistent with the longer biological half-life ($T_{1/2}$) in RBCs as compared to plasma (see Table 3-1). No data on protein binding in the plasma were available. These data suggest that THF-associated radioactivity partitions rapidly to the plasma, resulting in higher peak concentrations in the plasma than in RBCs.

Total recovery of the administered dose in tissues was minimal, ranging from 3.7 to 10.3% in male and female rats of both dose groups. The highest percent recovery was in the carcass, indicating that THF or its metabolites are widely distributed. Tissue-specific data on a concentration basis (μg equivalent THF/g tissue) are shown in Table 3-3. These data indicate that the liver has the highest concentrations of radioactivity, followed by the fat, and then adrenal glands. Both male and female rats had similar patterns in the tissue distribution of THF-associated radioactivity at the two treatment doses, suggesting that at doses between 50 and 500 mg/kg, no significant shift in relative target tissue doses would be expected.

Table 3-3. Radiolabel concentration in tissues of rats and mice at 168 hours following gavage administration of [^{14}C]-THF

	Rat		Mouse		Rat		Mouse	
	Male	Female	Male	Female	Male	Female	Male	Female
	Dose: 50 mg/kg				Dose: 500 mg/kg			
Tissue	Tissue concentration (μg equivalent/g)							
Carcass	2.0	1.5	1.4	0.9	11.9	8.8	14.2	12.4
Skin	2.4	1.6	1.5	0.9	14.7	7.4	18.1	14.6
Whole blood	1.0	0.7	0.8	0.5	6.1	5.1	8.6	5.5
Bone marrow	3.7	2.9	1.1	2.4	17.0	9.4	0.2	9.9
Brain	2.1	1.3	1.4	1.0	8.3	7.7	12.3	10.0
Fat	4.1	3.0	3.1	2.2	31.3	14.0	35.7	20.5
RBCs (terminal)	1.8	1.2	1.2	0.9	8.5	8.1	12.8	8.8
Heart	1.7	1.4	1.0	0.8	10.1	7.8	11.6	9.0
Lungs	2.1	1.4	1.1	0.6	11.9	7.9	11.6	8.6
Spleen	2.2	1.1	1.0	0.7	9.5	6.6	12.9	9.1
Liver	15.4	11.9	1.4	0.9	60.5	38.3	17.9	12.9
Kidney	2.7	2.0	1.7	1.1	15.8	12.2	22.8	14.1
GI tract	1.8	1.0	0.9	0.6	8.4	6.0	11.3	8.0
GI contents	0.5	0.2	0.2	0.1	1.3	0.9	1.5	1.2
Ovaries	–	1.4	–	1.1	–	8.4	–	13.0
Testes	1.8	–	1.4	–	7.3	–	12.5	–
Adrenals	5.4	3.9	3.0	1.4	30.2	18.5	27.1	23.5
Plasma (terminal)	0.6	0.3	0.3	0.2	3.4	2.2	3.1	4.3
Uterus	–	1.1	–	0.8	–	7.8	–	8.5
Muscle	2.0	1.7	1.3	1.0	11.5	10.3	12.5	9.7
Bone	1.8	1.2	1.2	0.6	10.6	7.5	8.3	6.3

Source: Adapted from DuPont Haskell Laboratory (1998).

Similar to rats, THF-associated radioactivity appeared rapidly in the plasma of mice after oral exposure. Evaluation of kinetic parameters for blood compartments showed that peak concentrations were higher, but total integrated doses (AUC) were lower in plasma compared to RBCs. In mice, the total percent of the administered dose recovered within 168 hours after oral dosing in these tissues ranged from 3.1 to 4.0%. The highest percent of the dose was recovered in the carcass, indicating that THF or its metabolites were widely distributed. Tissue-specific data on a concentration basis (μg equivalent THF/g tissue) at 168 hours are shown in Table 3-3. Tissue distribution of THF-associated radioactivity was reported for male mice at multiple time points until terminal sacrifice at 168 hours after dosing. In the high-dose males, peak concentrations were reached within 4 hours after dosing for all of the tissues studied, with peak concentrations notably higher in the adrenal glands, liver, and kidney. The rate of decrease in the levels of radioactivity was tissue dependent. Most notably, at longer time points, fat had higher levels of radioactivity than liver. At the low dose, the peak concentrations of radioactivity in the liver and kidney, but not adrenal glands, were higher than in other tissues. As in the high-dose group, the concentration of radioactivity in the fat of the low-dose group at 168 hours was higher than in other tissues measured.

Hara et al. (1987) investigated the distribution of THF by giving 300 and 700 mg/kg THF orally to male Wistar rats and rabbits (strain unspecified), respectively. Blood and tissue samples were collected for analysis of THF concentrations from groups of three rats at 10 and 30 minutes and at 1, 2, 3, and 5 hours. Blood and tissue samples were also collected for analysis of THF from two rabbits at 7 or 8.5 hours after administration. No significant differences were observed between the two species. Ratios of tissue levels to blood levels were approximately 1.5–2.0 in adipose tissue and kidney and about 1.0 in the brain, liver, spleen, and muscle for both rats and rabbits.

The distribution of THF has also been studied following inhalation exposures in animals. Elovaara et al. (1984) measured the distribution of THF into the brain and fat tissue of rats exposed to 0, 200, 1,000, or 2,000 ppm (0, 590, 2,950, and 5,900 mg/m^3) THF 6 hours/day, 5 days/week for 2–18 weeks. The exposed rats were sacrificed at 2, 8, 13, or 18 weeks, and THF concentrations were measured in the brain and perirenal fat. At all of the time points, THF concentrations in the fat were consistently higher than in the brain by a factor of approximately two- to threefold. THF in both tissues increased with THF exposure concentration. As the treatment extended from 2 to 18 weeks, the THF concentrations in both tissues gradually decreased. The authors suggested that the decrease in tissue levels with longer exposure duration was due to induction of the oxidative metabolism of THF, as evidenced by increases in 7-ethoxycoumarin O-deethylase activity (as a marker for metabolic enzyme activity) in the liver and kidney of THF-exposed animals beginning at 2 weeks (not duration-dependent). However, the observed statistically significant increases in enzymatic activity appeared to reflect a decrease in the activity in control animals rather than an increase in activity in the treated animals. No changes in liver cytochrome P450 (CYP450) content were observed at the end of the study. Comparison with the highest exposure concentration showed that tissue levels of THF were greater than the 10-fold difference in dose. This result is consistent with the greater partitioning of THF as the parent compound into fatty tissues as discussed above for the oral dosing study in mice.

Kawata and Ito (1984) compared the distribution of THF following several different inhalation exposure regimens. Male Wistar rats (5/control group and 25/experimental group) were exposed to 15,000 ppm (44,250 mg/m^3) THF for a single 30-minute exposure or for seven daily 30-minute exposures. In addition, rats were exposed to 3,000 ppm (8,850 mg/m^3) THF vapor for 1 hour/day, 5 days/week for 12 weeks. THF concentration was determined in tissues immediately and 1, 3, 6, and 12 hours following the last exposure. Tissues evaluated in the study were the brain, thymus, lung, heart, liver, kidney, spleen, and blood. For the single exposure group, immediately after exposure, the pattern of THF distribution in organs was: blood > brain = kidneys = heart > liver = spleen = thymus = lungs. Within 1 hour, differences among the tissue levels began to decrease, with only the lung levels being significantly lower and blood levels being significantly higher than the other tissues. No significant difference in THF levels was observed among the tissues within 3 hours postexposure. The study authors suggested that lower levels of THF in the lung reflected elimination of unmetabolized THF. Lower levels of THF in the liver and kidney would be consistent with the metabolic capacity of these organs, since THF was measured as the parent compound in this study. Repeated exposure to 15,000 ppm THF resulted in a similar pattern of tissue level, except that immediately after exposure only the lung (significantly lower) and blood (significantly higher) levels were different from the other tissues.

In the rats exposed to 3,000 ppm THF for 12 weeks, a different pattern of distribution was observed. Immediately after the last exposure, THF tissue levels were greatest in the thymus, followed by spleen > brain = heart > lung > blood > liver = kidney. The concentration of THF in thymus was significantly higher than THF concentration in other tissues and remained higher for up to 12 hours postexposure. Tissue levels of THF measured immediately after the last exposure for the 1-day and the 6- or 12-week 3,000 ppm exposure regimens were compared. THF levels were proportionally higher with increasing duration of exposure from 1 day to 6 weeks, although for many tissues THF levels at 6 weeks were similar to those observed at 12 weeks. Daily tissue accumulation was most apparent for the thymus, in which tissue concentrations were nearly twice as high as for the other tissues immediately after the last exposure at 12 weeks. Beginning at 6 weeks of exposure, THF concentrations were also notably higher in the spleen than in other tissues. Taken together, these data show that THF is taken up in the blood and is widely distributed following exposure by the inhalation route. Longer duration exposures may generate daily accumulation in some organs, although tissue levels decrease to background levels rapidly after cessation of exposure. THF distributed preferentially to the thymus and spleen following subchronic exposures. The study authors suggested that higher THF concentrations in the thymus after longer-term exposures might reflect increased age-associated fattening of the thymus periphery, which seems to coincide with the normal age-related atrophy in the parenchyma of this organ. However, the spleen was also noted as an organ with high tissue concentrations, suggesting to the study authors (Kawata and Ito, 1984) the possibility of THF distribution through the lymph system.

Pellizzari et al. (1982) reported the presence of THF in the milk from mothers who were living in one of four urban areas in the United States. THF was found in one of eight samples that were analyzed. This study did not provide quantitative data on the concentrations of THF that were present or information on mothers' exposure.

No data on placental transfer of THF or fetal distribution is available in humans or in animal studies.

3.3. METABOLISM

Several lines of evidence suggest that THF undergoes oxidative metabolism by liver microsomal CYP450 enzymes followed by further hydrolysis catalyzed by lactonase (also known as paraoxonase1 or PON1) and additional oxidation by cytosolic dehydrogenases. Based on the available in vivo and in vitro data, the ultimate metabolite of THF is CO_2 and the proposed metabolic pathway for this conversion is presented in Figure 3-1 (Couper and Marinetti, 2002; DuPont Haskell Laboratory, 2000). According to this pathway, THF undergoes oxidative metabolism to form the intermediates 5-hydroxy-THF and 4-hydroxybutanal which may undergo further oxidation to γ-butyrolactone (GBL), γ-hydroxybutyric acid (GHB), and succinaldehyde.

In vivo studies on THF metabolism indicate that CO_2 is the major terminal metabolite, as shown in Table 3-2 (DuPont Haskell Laboratory, 1998). In mice administered a single gavage dose of 50 mg/kg [^{14}C]-THF, the percent of the radioactivity recovered as CO_2 was 58.2% in males and 74.6% in females. Volatile organics (possibly as unmetabolized THF) accounted for 17.8% of the administered dose in males and 24.5% of the administered dose in females. In mice administered a single dose of 500 mg/kg [^{14}C]-THF, the percent of the administered dose recovered as CO_2 was 51.1 and 36.2% for males and females, respectively. Rat metabolism studies also demonstrated that oxidative metabolism of THF to CO_2 is an important pathway. In rats given a single gavage dose of 50 mg/kg of [^{14}C]-THF, 47.8 and 47.5% of [^{14}C]-THF in males and females, respectively, was recovered in the form of CO_2. In rats given 500 mg/kg of radiolabeled THF, these percentages were 21.9% in males and 18.8% in females.

In both sexes of mice and rats, metabolism of THF to CO_2 was greater at the low dose, suggesting that metabolism may be saturated at higher doses. Although the data suggest that there might be species differences in the contribution of CO_2 to THF metabolism, potential saturation of the CO_2 trap, and therefore loss of CO_2 in the rat study makes comparison of the rat and mice data unreliable.

The metabolism of GBL and GHB has also been studied extensively (NSF, 2003). GBL may readily convert to GHB, as lactones are known to readily equilibrate in aqueous media between their closed (lactone) and open (hydroxyl acid) forms, a process that may be influenced by pH and structural features of the specific lactone (Teiber et al., 2003; Roth and Giarman, 1966). Hydrolysis of lactones to the corresponding organic acids as well as the reverse reaction, namely formation of lactones from hydroxy acids, have recently been shown to be catalyzed by liver and serum enzymes known as paraoxonases (PON) (Draganov et al., 2005; Teiber et al., 2003; Billecke et al., 2000). In these studies, several lactone and hydroxy acid substrates, including GBL and GHB, were converted to the corresponding hydroxy acids and lactones by a specific human serum PON isoenzyme (PON1).

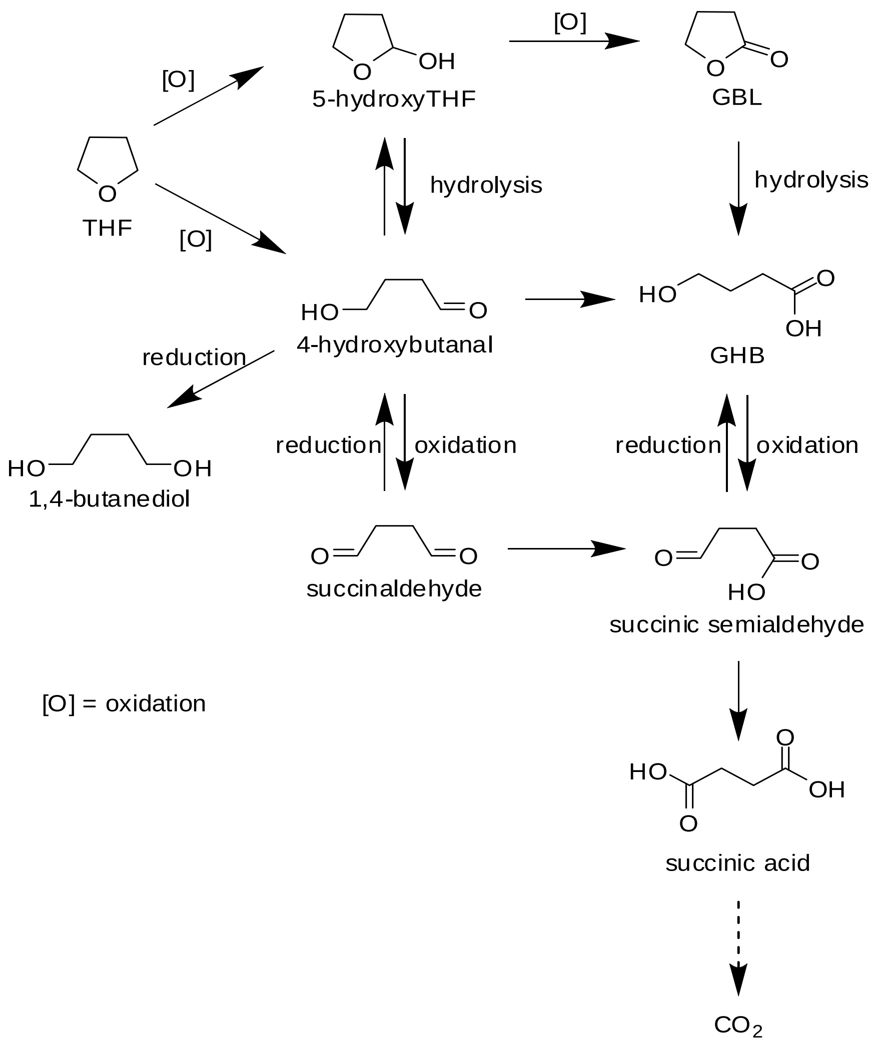


Figure 3-1. Possible metabolic pathways of THF.

Source: Modified from Couper and Marinetti (2002) and DuPont Haskell Laboratory (2000).

It is well established that, in the absence of exposure to THF, normal brain and peripheral tissues from several mammalian species, including humans, have built in metabolic machinery to produce and process GHB. High concentrations of GHB have been found in normal brain and in peripheral tissues including brown fat, liver, heart, spleen, and kidneys from human and other species where endogenous formation of brain GHB is thought to come from the neurotransmitter γ-aminobutyric acid (GABA) and possibly 1,4-butanediol (Nelson et al., 1981; Doherty et al., 1978; Roth and Giarman, 1968). More recently, a GHB receptor from a human brain frontal cortex cDNA library has also been cloned and characterized (Andriamampandry et al., 2007).

GHB can be oxidized to succinic semialdehyde (SSA) by a cytosolic $NADP^+$ dependent GHB dehydrogenase commonly found in brain as well as several other tissues including brown fat, liver, heart, spleen, and kidneys (Kaufman and Nelson, 1987; Kaufman et al., 1979). An enzyme known as succinic semialdehyde dehydrogenase then oxidizes SSA to succinic acid (Kaufman and Nelson, 1987; Gibson et al., 1983) which is an intermediate in the citric acid cycle that ultimately generates CO_2, water, and usable energy. As discussed earlier, the in vivo metabolism studies of THF have shown that CO_2 is the predominant metabolite.

In an in vitro experiment with hepatic microsomal preparations from rats, mice, or humans, the only metabolite of THF identified was GHB (DuPont Haskell Laboratory, 2000). The $T_{1/2}$ for disappearance of THF in these reactions was 40 hours for rat microsomes, 28 hours for human microsomes, and 9 hours for mouse microsomes. The data suggest that liver microsomes in mice may have a greater capacity to metabolize THF than do human or rat microsomes. No data are available to confirm whether these relative rates of metabolism by microsomes are predictive of THF metabolism among species in vivo. Further, though no attempt was made to characterize the role of specific metabolizing enzymes, the fact that microsomes were used, in the presence of an NADPH-generating system (DuPont Haskell Laboratory, 2000), strongly suggests that one or more of the CYP450 isoenzymes were involved.

The metabolism of THF to GBL is further supported by metabolic studies of p-dioxane, a structural analogue of THF. p-Dioxane-2-one, a lactone with a six-member ring analogous to GBL, has actually been identified as the major urinary metabolite of p-dioxane in rats (Woo et al., 1977). In addition, in vitro studies of structurally related compounds with a THF ring or similar ring structures indicate that there are a number of possible pathways (see Figure 3-1) for the metabolism of THF to GHB, including (1) α-hydroxylation (by microsomal CYP450 enzymes) to 5-hydroxy-THF, which can be rapidly converted to GBL and GHB (Woo et al., 1977; Fujita and Suzuoki, 1973); (2) oxidation of THF (by cytosolic enzymes) to 4-hydroxybutanal, followed by immediate oxidation to GHB and GBL or reversibly reduced to 1,4-butanediol (El Sayed and Sadée, 1983; Roth and Giarman, 1968); and (3) direct oxidation of THF to succinaldehyde (by microsomal CYP450 enzymes)—not shown in Figure 3-1, followed by reversible reduction to 4-hydroxybutanal and oxidation to GBL or GHB in the presence of cytosolic soluble enzymes. The formation of GBL or GHB from succinaldehyde by soluble enzymes could also occur by oxidation to SSA, followed by reversible reduction (El Sayed and Sadée, 1983).

The implication of these metabolic intermediates to the overall toxicity of THF is unclear. Many of these intermediates (i.e., 5-hydroxy-THF, 4-hydroxybutanal, 1,4-butanediol, succinaldehyde) are expected to be unstable and rapidly undergo further metabolism to GHB. Studies in rats have shown that 1,4-butanediol is metabolized in the blood and brain to GHB and that GHB is the active intermediate responsible for the central nervous system (CNS) effects of 1,4-butanediol (Roth and Giarman, 1968). In fact, in vitro and in vivo studies have shown that GHB can be converted to the neurotransmitter, GABA (Vayer et al., 1985; DeFeudis and Collier, 1970), which provides a possible mechanistic link between THF and its potential for causing CNS effects. Appreciable amounts of radioactive-labeled GABA were detected in the brains of mice 60, 120, and 180 minutes after intraperitoneal (i.p.) injection of 1-[^{14}C]-GHB (DeFeudis and Collier, 1970). Increased tissue level of GABA and putrescine (the primary source of GABA in many tissues) may also be hypothesized to play a role in the THF-induced cell proliferation and carcinogenicity in the liver (see Section 4.7.3.2).

3.4. ELIMINATION

The available human data suggest that expiration is an important route of excretion for THF. In a human occupational study (Ong et al., 1991), workers exposed to THF by the inhalation and dermal routes excreted THF in exhaled air and in the urine. Kageyama (1988) measured exhaled air concentrations of THF in volunteers exposed by the inhalation route. THF was present in the exhaled air for several hours after exposure to a concentration of 200 ppm, suggesting that THF is excreted in exhaled air. Droz et al. (1999) summarized the results from several additional human volunteer studies that support the conclusion that THF is rapidly excreted from the body via exhaled air and urine. Exposure periods were for as long as 8 hours to concentrations as high as 200 ppm. In all cases, THF levels in breath, blood, or urine declined rapidly and reached background levels within a period of approximately 12 hours.

Oral dosing studies in animals provide further evidence for the important role that exhaled air plays as a route of excretion for THF. In rats exposed to an oral dose of 50 mg/kg THF, 47% of the oral dose was recovered in the expired air as CO_2, while only about 4% of the radioactivity was detected in the urine and 1% in the feces. In the mice exposed to the same dose of THF, 58–75% of the oral dose was recovered in expired air as CO_2 and 18–25% as volatile organic compounds (VOCs), while 3–5% of the radioactivity was detected in the urine and 1% was detected in the feces. A similar pattern was observed in the animals exposed to the high dose of 500 mg/kg, but relatively less radioactivity, 19–22% as CO_2 in rats and 36–51% as CO_2 in mice, was recovered in the expired air. Because of some technical difficulties in recovery of

VOCs from the expired air, significant losses of trapped VOCs occurred in most of the measurements. Among all the data available for VOCs, the only adequate data were from the mice exposed to the low dose of THF. Nevertheless, the available data indicate that expiration was the major route of excretion of absorbed THF, and CO_2 was the major final product. The study authors suggested that the VOCs in the exhaled air were likely to be parent THF. Urine and feces were relatively minor routes of THF excretion (DuPont Haskell Laboratory, 1998).

In the DuPont Haskell Laboratory (1998) study, the time course of THF in the plasma of exposed rats and mice was also studied. The results are summarized in Table 3-1. In the rats exposed to the low dose (50 mg/kg), the $T_{1/2}$ of the radioactivity in the plasma was 52 hours in the males and 51 hours in the females. Following exposure to the high dose (500 mg/kg) THF, the plasma $T_{1/2}$ was estimated to be 48 (males) and 59 (females) hours. In the mice exposed to the low dose, the plasma $T_{1/2}$ was 57 hours for males and 51 hours for females. Following exposure to the high dose (500 mg/kg) THF, the serum $T_{1/2}$ was 57 (males) and 99 (females) hours. Based on these data, there were no apparent differences in the plasma $T_{1/2}$ between rats and mice. At the 50 mg/kg dose level, male and female animals had a comparable $T_{1/2}$, while at 500 mg/kg THF the males had shorter plasma half-lives than the females. The half-lives reported in this study are not the biological half-lives of THF but only represent radioactivity measured in plasma and serum. The radioactivity present is likely derivatives of THF that are either covalently bound to cellular macromolecules or have been incorporated into the primary carbon pool. Available data indicate that the biological $T_{1/2}$ of THF is about 5–7 hours. Hara et al. (1987) reported a $T_{1/2}$ of 5.2 hours in rats, following oral administration of 300 mg/kg, and a $T_{1/2}$ of 5.1 hours in rabbits at a dose of 700 mg/kg.

The AUCs for the THF-associated radioactivity in the plasma were estimated for the exposed rats and mice in the study conducted by the DuPont Haskell Laboratory (1998). In the rats exposed to 50 mg/kg THF, the plasma AUC in males and females was 536 and 320 μg THF equivalents-hour/g plasma, respectively. In rats exposed to 500 mg/kg THF, the plasma AUC in males and females was 2,826 and 1,998 μg THF equivalents-hour/g plasma, respectively (see Table 3-1). At either the low or high doses, the AUC was always higher in the male rats than in female rats. A similar difference between sexes was observed in mice. In the 50 mg/kg dose group, the plasma AUC was 207 (male) and 157 (female) μg THF equivalents-hour/g plasma. The plasma AUC in males and females was 3,238 and 1,904 in the high-dose group (500 mg/kg), respectively. Based on these findings, the same oral dose of THF resulted in a higher internal dose of THF and/or its metabolites in male rats or mice than in females of the corresponding species. However, the toxicological implications of this result are difficult to interpret since the AUC reflects a combination of THF and its metabolites, while the toxic moiety has not been identified. Nevertheless, in general, the greater AUC for males would be consistent with a greater degree of systemic dose in males versus females.

The AUC data from this study can be used to estimate the body clearance of THF. The clearance was calculated based on the ratio of administered dose/AUC. All the relevant kinetic parameters and estimated clearance values are summarized in Table 3-1. In both rats and mice, females had a higher clearance rate than males. The more rapid clearance (i.e., due to lower AUC values) observed in females might reflect differences in excretion kinetics or alternatively might reflect differences in the degree of THF absorption, since the administered dose was used for this calculation rather than the absorbed dose. The clearance rates in rats of the low-dose group were lower than the high-dose group, while there was no such variance in mice.

Kawata and Ito (1984) compared the blood and tissue distribution and elimination of THF, following several different inhalation exposure regimens. In male Wistar rats exposed to 15,000 ppm (44,250 mg/m^3) THF for a single 30-minute exposure, 70–80% of the THF was eliminated from the organs within 1 hour following exposure. After 1 hour, concentration of THF decreased slowly and was almost completely eliminated by 12–13 hours following exposure. In animals that received seven exposures of 15,000 ppm, only 18–39% of THF was eliminated from the organs in 1 hour following exposure, indicating some saturability in the elimination kinetics for these organs at very high concentrations. In these animals, the rate of THF decrease was 31% at 3 hours following last exposure and 68% at 6 hours following last exposure; by 12 hours THF was almost completely eliminated. Similar to the acute dosing studies, THF was nearly completely eliminated from blood and tissues within 12 hours after the last exposure in the 12-week exposure protocol. These data indicate that, for exposure concentrations as high as 15,000 ppm, THF is rapidly eliminated from blood and other tissues.

3.5. BIOACCUMULATION

Two toxicokinetic studies employed longer-term exposure regimens that provide information useful for assessing the potential for bioaccumulation of THF in tissues. Kawata and Ito (1984) measured tissue levels of THF immediately after the last exposure period following daily inhalation exposures to 3,000 ppm THF for 1 day, 6 weeks, or 12 weeks. Daily levels increased in some tissues, particularly from 1 day to 6 weeks. In the thymus and spleen, tissue levels continued to increase through the 12-week exposure period. These data suggest some potential for tissue accumulation with repeated daily exposure. However, it is notable that even in animals exposed for 12 weeks, tissue levels declined rapidly after the end of the last exposure period (within hours). Thus, the rate of uptake of THF is likely more rapid than the rate of excretion. Therefore, during periods of continuous exposure, there is some potential for tissue levels of THF to accumulate. However, periods of intermittent exposure would allow for clearance of the THF body burden and thus limit the potential bioaccumulation.

Elovaara et al. (1984) measured the distribution of THF into the brain and fat tissue of rats exposed to 0, 200, 1,000, or 2,000 ppm (0, 590, 2,950, and 5,900 mg/m^3) THF 6 hours/day, 5 days/week for 2–18 weeks. As the treatment extended from 2 to 18 weeks, the THF concentrations in both tissues of the exposed rats gradually decreased. The observed decline in brain and fat THF levels suggests that THF may not bioaccumulate in these tissues.

Evaluation of human volunteer studies to derive a physiologically based pharmacokinetic model for THF revealed rapid elimination of THF from the body (Droz et al., 1999). The resulting model predicted that no significant accumulation of THF would be expected over the workweek or across workweeks. THF elimination rates observed in inhalation (Elovaara et al., 1984; Kawata and Ito, 1984) and oral studies (DuPont Haskell Laboratory, 1998) in animals support this conclusion. Taken together, the data support the general conclusion that THF is not likely to bioaccumulate.

3.6. PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELS

A human physiologically based pharmacokinetic (PBPK) model has been developed by Droz et al. (1999) to estimate THF concentrations in the blood, breath, and urine, following an inhalation exposure for the purpose of determining biological exposure indices in these media that would equate to an occupational exposure level of 200 ppm THF. The PBPK model was constructed with seven compartments: lungs, muscles and skin, fatty tissue, liver, kidneys, brain, and other tissues. Physiological parameters (tissue volumes, blood flow rates, etc.) were calculated from body weight and height and from physical workload by using formulas previously developed by the author (Droz et al., 1989). Blood-air and tissue-air partition coefficients were estimated from in vitro experiments. THF metabolism was assumed to follow first order kinetics. Urinary excretions were calculated assuming a urine flow of 1 mL/min and a creatinine excretion rate of 1.4 g/day. The model was validated by using four discrete sets of human exposure data from workers or human volunteer studies. The model provided an adequate fit to three out of four data sets. The reason for the lack of fit for one of these data sets was not determined. Based on the model predictions, repeated inhalation exposures to 200 ppm THF would yield end-of-the-work-shift levels of THF in biological samples of 5.1 ppm in breath, 57 μmol/L in blood, and 100 μmol/L in urine. However, this model does not account for the toxicokinetic variability in humans and no PBPK models have been developed in animals. Also, there are no comparative toxicokinetic or toxicodynamic studies following exposure to THF by the oral route in humans and animals. Therefore, this model is not adequate for calculating human equivalent exposure concentrations from the available rodent study data.

3.7. SUMMARY

Overall, the available data demonstrate that THF is readily absorbed through multiple routes, is systemically distributed, and is rapidly metabolized and excreted. THF is readily absorbed from the respiratory tract, based on the observed rapid increase of THF in biological samples or calculated uptake rates in human studies (Droz et al., 1999; Ong et al., 1991; Kageyama, 1988; Wagner, 1974). Although no human data are available to evaluate the rate or degree of absorption of THF following exposure through the oral route, oral dosing studies in rats and mice show that radiolabeled THF is readily absorbed from the GI tract with wide tissue distribution; however, total recovery of radioactivity in tissues represented only a small fraction of the administered dose (DuPont Haskell Laboratory, 1998). No studies on dermal absorption were identified, but the observed systemic toxicity in a dermal toxicity study in mice and rabbits (Stasenkova and Kochetkova, 1963) demonstrated that THF can be absorbed through the skin.

A metabolic pathway has been proposed in which THF is oxidatively metabolized to succinic acid, which being an intermediate in the citric acid cycle, undergoes a series of reactions ultimately leading to the release of CO_2 from the parent molecule. In addition, several intermediate metabolites are expected to be unstable and rapidly undergo further metabolism to GHB which can be converted to the neurotransmitter GABA. Several enzymes, including CYP450, PON1, and dehydrogenases, may be involved in metabolizing THF and some of its intermediate metabolites (see Section 3.3 and Figure 3.1).

The available human data suggest that THF is rapidly excreted. Excretion in exhaled air and urine were correlated with exposure concentration in an occupational study (Ong et al., 1991). Human volunteer studies demonstrate that THF is rapidly excreted in exhaled air and urine, with concentrations of THF in these tissues generally returning to background levels within hours of cessation of exposure (Droz et al., 1999; Kageyama, 1988). The rapid excretion of THF observed in human studies is supported by an inhalation study in rats (Kawata and Ito, 1984) in which tissue levels of THF decline rapidly during the postexposure period. THF is also rapidly cleared from the body following oral dosing, with exhaled air serving as the primary route of excretion (DuPont Haskell Laboratory, 1998). Analysis of the mass balance of radioactivity in the exhaled air, excreta, and tissues showed that nearly the entire administered dose was excreted in the exhaled air as CO_2 or volatile organics (possibly unmetabolized THF). The rate of excretion was rapid. The half-lives in the plasma were approximately 50 hours for most groups, although blood and tissue levels of radioactivity decreased rapidly, and tissue levels of radioactivity represented only a small percentage of the administered dose within 168 hours of exposure. Available data indicate that the biological $T_{1/2}$ of THF is about 5–7 hours (Hara et al., 1987).

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS—EPIDEMIOLOGY, CASE REPORTS, CLINICAL CONTROLS

There are a number of occupational exposure studies and case reports on humans exposed to THF that identify effects on the nervous system and liver. Most of these studies do not identify THF exposure levels and all of them report coexposures to other chemicals, including neurotoxic solvents.

Garnier et al. (1989) reported two cases of occupational exposure to THF. In both cases, the men (ages 35 and 55) worked as plumbers repairing pipes in confined spaces with a glue that contained THF. No exposure information was provided. Symptoms included nausea, headache, dizziness, chest pain, cough, dyspnea, and epigastric pain. In both men, blood count and renal function were normal. However, the serum liver enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), and γ-glutamyl transferase (GGT) were elevated several times above the normal range. Clinical symptoms resolved in about 2 days and liver enzymes returned to normal within 2 weeks. The authors suggested that THF exposure may result in irritation, CNS effects, and transient liver toxicity in humans.

Emmett (1976) reported the case of a 41-year-old pipe fitter exposed for about 3 months to a mixture of THF and other solvents in a pipe cleaning solution and a pipe glue. Other solvents present in the solution included acetone and cyclohexanone. No information was provided on exposure concentrations. The only effects reported by the patient were a slight rhinorrhea (runny nose) during exposure and a gradual onset, over 10 weeks, of a constant unpleasant smell or loss of sense of smell. No other clinical signs were reported. A neurological exam, radiography of skull and sinuses, and hematological exam were all normal. Within 6 weeks after cessation of exposure, some sense of smell returned. However, by 7 months after the initial diagnosis, sense of smell was still diminished.

Edling (1982) reported the occupational exposure of a shoemaker to a mixture of solvents that included THF, acetone, chloroform, and trichloroethylene. No information on exposure concentrations was provided. In addition, the patient had concurrent exposure to acetylsalicylic acid to treat lumbago (back pain). Clinical chemistry results revealed increased liver enzymes including GGT and ALT. Liver biopsy showed centriacinar fatty change and siderosis.

Juntunen et al. (1984) reported cerebral convulsions in a patient following occupational exposure to both THF and enfluran anesthesia. The patient was a 45-year-old man who worked as a plumber, using a solvent containing THF to insulate the inside of a water piping system. For 2 weeks, the patient had been working with THF in enclosed spaces with no ventilation. No information was provided on the resulting exposure concentration. The patient reported that he had felt unusually tired and had a headache in the week before he was admitted to the hospital for an appendectomy. On awakening from the enfluran anesthesia, the patient had several convulsions. In addition, liver enzymes were slightly elevated following the surgery. The authors concluded that THF exposure was the main contributing factor for the convulsions because the patient was exposed to high concentrations of THF for 2 weeks before the surgery. In addition he had never had epilepsy or neurological disease and his clinical status and computed tomography results were normal.

Albrecht et al. (1987) reported a case of autoimmune glomerulonephritis in a plumber working with pipe cement containing THF. The 28-year-old male plumber had been working with pipe cement for over 9 years. The initial symptom was gross hematuria. A needle biopsy of the kidney revealed segmental proliferative glomerulonephritis with immunoglobulin A deposits, capillary adhesions to the Bowman's capsule, and fibrin in the glomerular mesangial deposits. Industrial hygiene monitoring identified 15-minute exposures to THF, ranging from 389–757 ppm (1,148–2,233 mg/m^3) during periods that pipe cement was in use.

The National Institute for Occupational Safety and Health (NIOSH) (1991) investigated reports of adverse health effects at a plant that manufactured flexible hose. Environmental monitoring was conducted for respirable particulates, respirable silica, THF, total dust, metals, nitrosamines, and other organic compounds. Approximately 35–40 employees were interviewed by NIOSH investigators. In addition, the medical records of six employees who had sought medical attention for a work-related health problem and the death certificates of nine employees who were thought to have had work-related health problems were reviewed by NIOSH investigators. THF was detected in five air samples collected during a sealing operation. The concentrations ranged from 20 to 83 ppm (59–245 mg/m^3), but none of the sampling results were above the Occupational Safety and Health Administration standard of 200 ppm. However, the backup sections on the sampling apparatus also contained THF, indicating that breakthrough had occurred and suggesting that the THF exposure concentrations may have been higher. In addition to THF, other organic solvents detected in the air monitoring samples included acetone, toluene, methyl ethyl ketone, and 1,1,1-trichloroethane. The interviewed employees reported a variety of symptoms, including eye and respiratory tract irritation, headaches, lightheadedness, and drowsiness. The authors suggested that these symptoms may be related to solvent exposure but could not associate specific symptoms with individual chemicals.

Horiuchi et al. (1967) evaluated the health of workers employed in a vinyl chloride hose-manufacturing facility where THF was used as an adhesive. THF was detected in workplace air samples at concentrations as high as 1,000 ppm (2,950 mg/m^3). Workers who handled THF reported fatigue in the lower extremities. Clinical findings included decreased specific gravity of whole blood (more predominant in females), decreased white blood cell count, increased serum ALT activity, palpable liver, and hypotension.

Two human dermal THF exposure studies were identified. A study by BASF (1938) did not observe contact dermatitis or sensitization in dermal tests in 196 volunteers exposed to THF (exposure concentration not reported by study authors). Hofmann and Oettel (1954) reported that THF applied to the skin of six people produced irritation that was more severe when THF was allowed to evaporate. The authors concluded that THF itself was nonirritating, and the irritation was caused by impurities that remained after THF had evaporated away. No additional information was provided to evaluate the adequacy of this study.

4.2. SUBCHRONIC AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS—ORAL AND INHALATION

4.2.1. Subchronic Studies

4.2.1.1. *Oral*

No subchronic studies in animals by the oral route of exposure were identified.

4.2.1.2. *Inhalation*

Horiguchi et al. (1984) evaluated the subchronic inhalation toxicity of THF in rats. Male Sprague-Dawley rats (11–12/group) were exposed to THF vapors 5 days/week, 4 hours/day for 12 weeks. Two experiments using different concentrations were conducted. THF concentrations for the first experiment were 0, 200, or 1,000 ppm (0, 590, or 2,950 mg/m^3) and for the second experiment were 0, 100, or 5,000 ppm (0, 295, or 14,750 mg/m^3). Body weights and clinical signs of intoxication were observed daily during the exposure period. Rats were sacrificed on the second day following termination of exposure. Blood was drawn for hematological and serum chemistry evaluation. Major organs were weighed and evaluated histopathologically. Body weight in rats exposed to 5,000 ppm was significantly lower than controls for the entire exposure period; no differences from controls were observed in the other treated groups. Animals in the 5,000 ppm group displayed signs of local irritation and CNS effects, which were described by the study authors as similar to those observed for the acute study (Horiguchi et al., 1984). These local irritation and CNS effects were reported as moderating with continued exposure. There were statistically significant increases in serum AST at exposures ≥200 ppm; however, the magnitudes of the increases were minimal and were not dependent on the exposure levels (the highest increase was 50% greater than controls at 1,000 ppm while at 5,000 ppm it only increased by 18%). Compared to the control values, the following parameters were also changed in the 1,000 and/or 5,000 ppm exposure groups. At 1,000 and 5,000 ppm, cholinesterase was slightly but significantly increased by 8 and 15%, respectively, while blood sugar was significantly decreased by 20 and 39%, respectively. Serum ALT, cholesterol, and bilirubin were significantly increased only in the 5,000 ppm group (by 100, 44, and 46%, respectively). White blood cell count was significantly decreased (by about 24%) in the 5,000 ppm group compared with controls. Relative organ weights were significantly increased (by 7–28%) only in the 5,000 ppm group, including brain, lung, liver, pancreas, and kidney, while the relative spleen weight was decreased (by 13%). All histopathological findings were comparable between treated and control groups. Based on body weight, organ weight changes, local irritation and CNS effects, and serum chemistry parameter changes, EPA identified 5,000 ppm (14,750 mg/m^3) as the study lowest-observed-adverse-effect level (LOAEL) and the no-observed-adverse-effect level (NOAEL) as 1,000 ppm (2,950 mg/m^3). The results of Horiguchi et al. (1984) were also reported in an earlier Japanese publication from the same laboratory (Katahira et al., 1982).

In an NTP subchronic inhalation study (NTP, 1998; Chhabra et al., 1990), F344/N rats and B6C3F$_1$ mice (10/sex/group) were exposed to target concentrations of 0, 66, 200, 600, 1,800, or 5,000 ppm THF vapor (0, 195, 590, 1,770, 5,310, or 14,750 mg/m^3) 6 hours/day, 5 days/week for 90 days. Animals were observed for morbidity and mortality, body weight, and clinical observations. Within 24 hours after last exposure, animals were euthanized, and blood and tissues were collected. All major tissues were fixed in formalin and processed. Histopathological examination was performed on all tissues from the high-concentration group and controls and on all gross lesions and target tissues from all exposure groups. Organ weights were measured for heart, liver, lung, right kidney, spleen, and thymus. Standard hematology and clinical parameters were evaluated in rats only. Thymus and liver weights and relative weights are summarized in Table 4-1.

Table 4-1. Changes in absolute and relative thymus and liver weights of F344/N rats and B6C3F$_1$ mice following subchronic inhalation exposure to THF[a]

	Concentration (ppm)					
	0	66	200	600	1,800	5,000
Male rats						
Body weight (g)	361 ± 6	353 ± 7	368 ± 11	364 ± 6	372 ± 9	343 ± 7
Thymus weight (g) Relative weight (mg/g)	0.36 ± 0.02 1.00 ± 0.03	0.35 ± 0.01 1.00 ± 0.03	0.33 ± 0.01 0.92 ± 0.015	0.35 ± 0.01 0.95 ± 0.04	0.33 ± 0.02 0.88 ± 0.03[b]	0.28 ± 0.02[c] 0.81 ± 0.04[c]
Liver weight (g) Relative weight (mg/g)	12.65 ± 0.65 34.92 ± 1.34	11.50 ± 0.49 32.53 ± 0.85	12.46 ± 0.41 33.84 ± 0.29	12.40 ± 0.42 34.04 ± 0.79	12.91 ± 0.38 34.72 ± 0.45	12.80 ± 0.31 37.28 ± 0.57[b]
Female rats						
Body weight (g)	205 ± 5	207 ± 5	205 ± 4	210 ± 3	209 ± 4	214 ± 3
Thymus weight (g) Relative weight (mg/g)	0.27 ± 0.01 1.29 ± 0.04	0.26 ± 0.02 1.26 ± 0.06	0.26 ± 0.01 1.26 ± 0.04	0.25 ± 0.02 1.17 ± 0.06	0.26 ± 0.01 1.25 ± 0.04	0.21 ± 0.01[c] 0.99 ± 0.03[c]
Liver weight (g) Relative weight (mg/g)	6.62 ± 0.13 32.36 ± 0.81	6.43 ± 0.17 31.05 ± 0.69	6.32 ± 0.19 30.76 ± 0.59	6.63 ± 0.22 31.52 ± 1.08	6.71 ± 0.19 32.02 ± 0.54	7.78 ± 0.17[c] 36.41 ± 0.87[c]
Male mice						
Body weight (g)	36.7 ± 0.8	36.9± 0.4	35.8 ± 0.7	36.3 ± 0.7	36.6 ± 0.8	32.7 ± 1.0[c]
Thymus weight (g) Relative weight (mg/g)	0.047 ± 0.003 1.27 ± 0.06	0.045 ± 0.003 1.23 ± 0.08	0.042 ± 0.002 1.17 ± 0.05	0.039 ± 0.001[b] 1.08 ± 0.05[b]	0.036 ± 0.003[c] 0.99 ± 0.07[c]	0.027 ± 0.002[c] 0.81 ± 0.05[c]
Liver weight (g) Relative weight (mg/g)	1.613 ± 0.037 44.00 ± 0.57	1.667 ± 0.022 45.24 ± 0.27	1.695 ± 0.037 47.28 ± 0.37[c]	1.722 ± 0.031[b] 47.52 ± 0.60[c]	1.789 ± 0.035[c] 48.94 ± 0.81[c]	1.964 ± 0.060[c] 60.03 ± 0.33[c]
Female mice						
Body weight (g)	32.4 ± 1.0	32.2 ± 0.6	33.3 ± 1.1	32.5 ± 0.7	33.1 ± 1.1	33.3 ± 1.1
Thymus weight (g) Relative weight (mg/g)	0.051 ± 0.003 1.57 ± 0.09	0.055 ± 0.003 1.71 ± 0.08	0.056 ± 0.002 1.71 ± 0.10	0.053 ± 0.002 1.64 ±0.06	0.052 ± 0.003 1.59 ± 0.11	0.046 ± 0.003 1.36 ± 0.08
Liver weight (g) Relative weight (mg/g)	1.592 ± 0.036 49.38 ± 0.94	1.574 ± 0.035 48.95 ± 0.92	1.609 ± 0.034 48.66 ± 1.30	1.551 ± 0.034 47.79 ± 0.60	1.733 ± 0.045[b] 52.51 ± 1.22[b]	1.814 ± 0.074[c] 54.42 ± 0.96[c]

[a]Organ weights and relative organ weights are, respectively, in g and mg organ weight/g BW (mean ± standard error). All group sizes are 10 animals/group except for male mice in the 5,000 ppm group where N = 7.
[b]$p \leq 0.05$.
[c]$p \leq 0.01$.

Source: Adapted from NTP (1998).

In F344/N rats, body weight and survival were not affected by THF exposure. Immediately after exposure, clinical signs of ataxia, described as irregular movement with lack of coordination, were observed in both male and female rats at 5,000 ppm only. In male and female rats at 5,000 ppm, absolute and relative thymus (Table 4-1) and spleen weights were statistically significantly decreased. In the 5,000 ppm exposure group, there were statistically significant increases in absolute and relative liver weights of female rats (by 17 and 13%, respectively) and in relative weights of male rat liver (by 7%), kidney (by 8%), and lung (by 15%). Several hematological parameters in both male and female rats were significantly increased at 5,000 ppm, including RBC counts, hemoglobin, volume of packed red cells, mean corpuscular volume, mean corpuscular hemoglobin (males only), segmented neutrophil count (males only), and platelet counts (females only). In the 5,000 ppm exposure group, male and female rats had increased levels of serum bile acids (by 70 and 80%, respectively) but the increase was statistically significant only in females; blood urea nitrogen and creatinine were also significantly decreased (by about 20%) in females. In the absence of cholestatic injury or hepatocellular necrosis (both alkaline phosphatase and ALT were normal) the change in bile acids was considered consistent with decreased or altered hepatocellular function (NTP, 1998). The only histopathological lesions observed in rats occurred in the forestomach at 5,000 ppm. Acanthosis (increased thickness) was found in 5/10 males and 8/10 females, and suppurative inflammation of the forestomach was found in 2/10 males and 4/10 females. However, the authors concluded that forestomach lesions were minimal inflammatory changes resulting from direct contact of THF ingested during the exposure period, rather than a systemic effect of inhaled THF. Based on observation of clinical signs, changes in organ weights, hematological effects, and clinical chemistry findings, EPA identified the concentrations of 5,000 ppm (14,750 mg/m^3) as a LOAEL and 1,800 ppm (5,310 mg/m^3) as a NOAEL in F344/N rats.

In B6C3F$_1$ mice, body weights were similar across groups, except for an 11% decrease in high concentration males. Survival in female mice was not affected by THF exposure for 14 weeks, while three high-concentration males died in weeks 2, 4, or 8 (NTP, 1998). Two male deaths were attributed to suppurative pyelonephritis, while the third (in week 4) was not explained. Male and female mice at both 1,800 and 5,000 ppm showed clinical signs of CNS toxicity characterized as narcosis during exposure. At 5,000 ppm, mice were in a stupor for 2 hours following the exposure period; at 1,800 ppm, mice were fully awake when chamber doors were opened following exposure. However, no incidence data were reported for CNS effects. In male mice, concentration-related trends included increasing relative liver weight starting at concentrations of 200 ppm (7.5% above control, $p < 0.05$) and both absolute and relative liver weights were significantly increased by 7–36% at concentrations of ≥600 ppm (Table 4-1). In addition, absolute and relative thymus weights were concentration-dependently decreased (by 15–36%) at concentrations of ≥600 ppm. Absolute and relative spleen weights were significantly decreased (by 31–38%) at 5,000 ppm only (not shown). In female mice, absolute and relative liver weights were significantly increased (by 6–14%) at 1,800 and 5,000 ppm (Table 4-1). Absolute and relative weights of spleen, lung, and heart were all significantly decreased at 5,000 ppm (not shown). Histopathological lesions in mice were observed in liver, uterus, and adrenal gland (Table 4-2).

Table 4-2. Incidences of selected non-neoplastic lesions in $B6C3F_1$ mice following subchronic inhalation exposure to THF[a]

	Concentration (ppm)					
	0	**66**	**200**	**600**	**1,800**	**5,000**
Male mice						
Liver						
Cytomegaly, Centrilobular	0	–[b]	–	–	1 (1.0)[c]	7[d] (2.0)
Female mice						
Adrenal Cortex						
Degeneration, X-zone	0	–	–	–	0	10[d] (2.0)
Liver						
Cytomegaly, Centrilobular	0	–	–	–	0	10[d] (1.0)
Uterus						
Atrophy	0	–	–	–	0	10[d] (2.0)

[a]All examined group sizes are 10 animals/group.
[b]Tissue not examined.
[c]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.
[d]Significantly different ($p \leq 0.01$) from the control group by the Fisher's exact test.

Source: Adapted from NTP (1998).

Liver centrilobular cytomegaly was observed in 7/10 male mice (graded mild) and 10/10 female mice (graded minimal) at 5,000 ppm (statistically significant) and 1/10 male mice (graded minimal) at 1,800 ppm. In addition, 10/10 female mice at 5,000 ppm demonstrated uterine atrophy and degenerative changes of the adrenal cortex. EPA identified the LOAEL for this study as 1,800 ppm (5,310 mg/m^3) based on statistically significant liver effects and clinical signs of toxicity (narcosis); the NOAEL is 600 ppm (1,770 mg/m^3).

BASF (Gamer et al., 2002; BASF, 2001a) evaluated a series of endpoints in male F344 rats (6/group plus 5/group) and female $B6C3F_1$ mice (10/group plus 5) in tissues for which THF-treated animals developed tumors in the NTP cancer bioassay (NTP, 1998). Animals were placed in one of three groups that were exposed 6 hours/day for either 5 consecutive days, 5 consecutive days followed by a 21-day observation period, or 20 consecutive days over a period of approximately 28 days. Test animals were exposed "nose only" to average THF concentrations of 0, 598, 1,811, or 5,382 mg/m^3 (0, 199, 604, or 1,794 ppm), corresponding to the concentrations used in the NTP (1998) cancer bioassay. Concentrations adjusted for continuous exposure were 0, 107, 323, or 961 mg/m^3. For the animals in each of the four concentration groups, a full necropsy was done, including histopathological evaluation of the kidney (rat), liver (mouse), and uterus (mouse). No clinical effects, body weight changes, kidney weight changes, or gross pathology related to THF exposures were reported for male rats. In the low-concentration group, no gross or histopathological effects were observed. No clinical effects or gross pathology changes related to the THF exposures were reported for female mice. In mice exposed for 5 days, absolute and relative uterus weights were decreased in the high-concentration group. In mice exposed for 5 days and followed for a 21-day recovery period, relative uterus weights were decreased (up to 21%) and appeared to decrease in a concentration-dependent manner, although this decrease was not statistically significant. In mice exposed for 20 days, statistically significant increases in absolute body weight (5%), absolute liver weight (11%), and relative liver weight (6%) were reported. The absolute and relative uterus weights were decreased by 11 and 15%, respectively. None of the uterus weight changes for any of the groups were statistically significant. No treatment-related histopathological effects were observed in the uterus at any concentration. Histopathological effects in the form of fatty phanerosis (unmasking of previously invisible fat in the cytoplasm), especially in zones 3 (centrilobular) and 2 (midzonal), were observed in the livers of mice exposed to THF for 5 days or 20 days, but not in mice that had 5 days of THF exposure followed by a 21-days recovery period. Specifically, the study authors reported that fatty phanerosis was present in 5/10 and 10/10 animals exposed for 5 consecutive days at the mid and high THF concentration, respectively. Similar fatty changes were also seen in livers from all mice that were exposed for 20 days to the high THF concentration. It should be noted that “fatty phanerosis” is an obsolete term (Popjak, 1945) and that “fatty infiltration,” “fatty degeneration,” or “fatty change” may be more appropriate to describe the morphological manifestation of altered fat metabolism of the parenchyma cells. The report indicated that there were no additional liver changes including cloudy swelling, vacuolar degeneration, or necrosis. Other histopathological changes in the high-concentration 5-day exposure group included a change in the hepatocyte cytoplasm to a more homogeneously eosinophilic appearance as compared with hepatocytes in control livers.

Kawata and Ito (1984) evaluated the health effects of THF following several different inhalation exposure regimens. Male Wistar rats (5/control group and 25/experimental group) were exposed to 15,000 ppm (44,250 mg/m^3) THF for a single 30-minute exposure or for seven 30-minute daily exposures. Another group of rats were exposed to 3,000 ppm (8,850 mg/m^3) THF vapor for 1 hour/day, 5 days/week for 12 weeks. Animals were observed for clinical signs and body weight. Blood was collected for serum chemistry analysis from animals exposed to 3,000 ppm only. The following tissues were collected for histopathology: brain, thymus, lung, heart, liver, kidney, and spleen. Animals exposed to 15,000 ppm developed clinical signs of face-washing, shaking head, and rubbing face with paws. These behaviors were weaker and had shorter duration compared with those observed in rats that received repeated exposures (either seven 30-minute exposures to 15,000 or 3,000 ppm for 12 weeks). In addition, rats receiving seven exposures to 15,000 ppm developed irritation of skin and mucous membranes as evidenced by severe salivation and nasal discharge. Rats exposed to 3,000 ppm for 12 weeks also developed irritation symptoms that were milder than those observed at 15,000 ppm. No effects on body weight were observed after either single or multiple exposures to 15,000 ppm. However, by the fourth week of exposure, rats exposed to 3,000 ppm had significantly reduced body weight compared with controls. Serum chemistry parameters were comparable between treated and control animals. No histopathological lesions were observed in either of the groups exposed to 15,000 ppm. In the animals exposed to 3,000 ppm, histopathological lesions were observed in both lungs and kidney. Papillary hyperplasia and catarrhal (inflammation of mucus membranes) degeneration were observed in lungs and bronchial epithelium. Protein casts and hyaline droplet degeneration were observed in the kidney tubule lumen epithelium in kidneys. Based on lung and kidney histopathological lesions, EPA identified 3,000 ppm (8,850 mg/m^3) as a LOAEL; a NOAEL was not established.

BASF (1938) investigated the subchronic effects of THF exposure in dogs. Four dogs (strain and sex not specified) were exposed by inhalation to THF vapor at a concentration of 200 ppm (590 mg/m^3) 6 hours/day, 5 days/week for 9 weeks, followed by exposure to a concentration of 366 ppm (1,080 mg/m^3) 6 hours/day, 5 days/week for 3 weeks. At the end of the 12 weeks, two of the four dogs were exposed on 2 successive days to a THF concentration of approximately 2,100 ppm (5,250 mg/m^3). Blood pressure was measured in each dog in the morning and afternoon for a 4-week pre-exposure control period and then before and after each daily exposure during the 12-week exposure period. Hematology, urinalysis, and limited pathological evaluations were also performed. Pulse pressure was decreased in 3/4 dogs following exposure to 200 ppm during weeks 3–4 of the study. In addition, increasing the THF concentration to 366 ppm resulted in a decrease in blood pressure compared to the control period in 3/4 dogs. In the two dogs exposed to 2,100 ppm THF, a “sharp drop” in systolic, diastolic, and pulse pressure was reported by the study authors after the second day of exposure. No signs of narcosis or eye or respiratory tract irritation were observed in these two dogs. In one dog, hemoglobin decreased and white blood cells increased compared to the control levels. However, examination of the urine did not reveal any abnormality in kidney function. No gross or microscopic pathology was observed in the heart, lungs, spleen, pancreas, or kidneys of any of the dogs. Based on alterations in blood pressure, the study authors (BASF, 1938) reported a LOAEL of 200 ppm (590 mg/m^3).

4.2.2. Chronic Studies and Cancer Bioassays

4.2.2.1. *Oral*

No chronic studies in animals by the oral route of exposure were identified.

4.2.2.2. *Inhalation*

Stasenkova and Kochetkova (1963) evaluated the effects of a 6-month inhalation exposure on rats. Male rats (20/group, strain not specified) in a single exposure group were exposed to air concentrations of 1–2 mg/L (1,000–2,000 mg/m^3) 4 hours/day, 7 days/week for 6 months. Endpoints evaluated included clinical signs, body weight changes, blood cell count, blood pressure, and functional condition of the neurovascular system, liver, and kidney. At the end of the 6-month treatment period, animals were sacrificed and histopathological examination of major organs was conducted. No effects were observed on behavior, body weight, liver and kidney function, or neuromuscular irritability of treated rats compared with controls. Within 2–3 months of treatment, exposed rats developed increased numbers of leukocytes, which remained elevated compared with controls for the remainder of the experimental period. After 3–4 months, blood pressure in treated rats was reduced compared to controls, and this observation continued for the remainder of the treatment period. Histopathological lesions included mild hypertrophy in the muscle fibers of the bronchi walls and arteries of lungs and spleen. Because of poor reporting of this study, no NOAEL or LOAEL can be identified.

NTP (1998) reported on the chronic toxicity and carcinogenicity of THF inhalation exposure in rats and mice. In the 2-year study, groups of F344/N rats and $B6C3F_1$ mice (50/sex/group) were exposed to 0, 200, 600, or 1,800 ppm (0, 590, 1,770, or 5,310 mg/m^3) THF 6 hours/day, 5 days/week for 105 weeks. Survival of treated rats was comparable to chamber controls at all exposure levels. Neither mean body weight differences nor clinical findings related to THF exposure were reported for either male or female rats. Pathology noted at sacrifice in male rats included apparent increases of renal tubular epithelial adenoma (at 600 and 1,800 ppm) and two renal tubular epithelial carcinomas (at 1,800 ppm), which, when combined with the adenomas, suggested a treatment-related trend. The incidences of adenoma or carcinoma in the 600 and 1,800 ppm males exceeded the historical range for chamber controls in the 2-year NTP (1998) inhalation studies, and the overall trend was statistically significant (p = 0.037). Table 4-3 summarizes the incidence of neoplastic and non-neoplastic changes in the kidney of male rats. No treatment-related changes in the incidence of neoplastic or non-neoplastic lesions in other tissues in the male or female rats were observed.

Table 4-3. Renal findings in male F344/N rats exposed to THF for 2 years

	Control	200 ppm	600 ppm	1,800 ppm
Number of animals examined	50	50	50	50
Nephropathy, chronic	48[a] (3.0)[b]	50 (2.9)	50 (3.1)	50 (3.0)
Hyperplasia	7 (3.4)[b]	5 (3.6)	6 (2.5)	7 (3.3)
Mineralization	8 (16%)[c]	7 (14%)	2 (4%)	5 (10%)
Adenoma	1/50 (2%)	1/50 (2%)	4/50 (8%)	3/50 (6%)
Carcinoma	0/50 (0%)	0/50 (0%)	0/50 (0%)	2/50 (4%)
Adenoma or carcinoma[d]	1/50 (2%)	1/50 (2%)	4/50 (8%)	5/50 (10%)
Adjusted rate[e]	8.3 %	16.7%	18.8%	38.3%
First incidence (days)	733 (T)[f]	733 (T)	631	668
Logistic regression test[g]	$p = 0.037$	$p = 0.602$	$p = 0.159$	$p = 0.065$

[a]Number of animals with lesions.
[b]Average severity of lesions in affected animals: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked.
[c]Percent affected.
[d]Historical incidence for 2-year inhalation studies with chamber controls: 6/652 (0.9 ± 1.3%); historical control range, 0–4%.
[e]Kaplan-Meier estimated tumor incidence at the end of the study, incorporating an adjustment for intercurrent mortality.
[f]T = terminal sacrifice.
[g]In the control column, the *p*-values are associated with the trend test. In the exposed group column, the *p*-values correspond to the pair-wise comparison between the controls and the exposed group.

Sources: Adapted from Chhabra et al. (1998); NTP (1998).

No treatment-related effects on survival or clinical observations were noted in female mice. Several statistically significant pathological changes were reported. These included concentration-related trends in hepatocellular adenoma or carcinoma (p <0.001). An increase in liver necrosis was also observed in females exposed to 1,800 ppm THF. Table 4-4 summarizes the incidence of neoplastic and non-neoplastic changes in the livers of female mice.

Table 4-4. Liver findings in female B6C3F$_1$ mice exposed to THF for 2 years

	Control	200 ppm	600 pm	1,800 ppm
Number of animals examined	50	50	50	48
Eosinophilic focus	7[a]	9	7	11
Necrosis	3 (2.0)[b]	0	0	7 (1.9)
Adenoma	12/50 (24%)[c]	17/50 (34%)	18/50 (36%)	31/48 (65%)
Logistic regression test[d]	*p* <0.001	*p* = 0.249	*p* = 0.188	*p* <0.001
Carcinoma	6/50 (12%)	10/50 (20%)	10/50 (20%)	16/48 (33%)
Adenoma or carcinoma[e]	17/50 (34%)	24/50 (48%)	26/50 (52%)	41/48 (85%)
Adjusted rate[f]	46.3%	61.3%	69.1%	93.0%
First incidence (days)	478	552	469	399
Logistic regression test	*p* <0.001	*p* = 0.188	*p* = 0.086	*p* <0.001

[a]Number of animals with lesions.
[b]Average severity of lesions in affected animals: 1= minimal; 2 = mild; 3= moderate; 4 =, marked.
[c]Percent affected.
[d]In the control column, the *p*-values are associated with the trend test. In the exposed group column, the *p*-values correspond to the pair-wise comparison between the controls and that of the exposed group.
[e]Historical incidence: 200/937 (21.3% ± 11.9%); range, 3–54%.
[f]Kaplan-Meier estimated tumor incidence at the end of the study, incorporating an adjustment for intercurrent mortality.

Sources: Adapted from Chhabra et al. (1998); NTP (1998).

In male mice, mean survival of the 1,800 ppm exposed group was significantly less than chamber controls (average life span of 456 versus 689 days). As a result, the number of male mice available for evaluation of neoplastic changes at the termination of the study was small (12 animals compared to 32 animals in the control group). The only clinical observation was narcosis in male mice exposed to THF at 1,800 ppm that lasted up to 1 hour following exposure. During periods of narcosis, the preputial fur was wet with urine, a condition that was thought to increase urogenital tract lesions and possibly lead to decreased survival. The lower survival rate and pathology findings, including bone marrow and lymph node hyperplasia, hematopoetic proliferation of the spleen, and thymic atrophy, were considered by the study authors (NTP, 1998) to be secondary to the urogenital tract inflammation. Although the number of male mice surviving to termination was small, statistical analyses for early mortality by NTP (1998) did not indicate that there was a treatment-related effect of THF on the incidence of liver tumors in male mice. Overall the only effect observed was clinical signs of toxicity (narcosis) in male mice at 1,800 ppm (5,310 mg/m^3).

Under the conditions of this 2-year bioassay, the NTP (1998) concluded that there was *some evidence* of carcinogenic activity of THF in male F344/N rats due to increased incidences of adenoma or carcinoma of the kidney and that there was *clear evidence* of carcinogenic activity of THF in female $B6C3F_1$ mice due to increased incidences of hepatocellular adenomas or carcinomas.

4.3. REPRODUCTIVE/DEVELOPMENTAL TOXICITY STUDIES—ORAL AND INHALATION

4.3.1. Oral

BASF (1994) reported the results of a one-generation reproductive toxicity range-finding study in rats given THF in drinking water. Male and female Wistar rats (10/sex/dose) were given THF at concentrations of 0, 4,000, 8,000, or 12,000 ppm in drinking water for 7 weeks prior to mating and throughout cohabitation, gestation, and lactation. THF intake values estimated from measured water consumption and body weights are shown in Table 4-5. The F0 females were allowed to litter and rear pups (F1 generation) for 4 days postpartum, at which time the litters were culled to eight pups/litter (ideally four of each sex). Culled pups were sacrificed and examined for gross pathologic lesions, and the surviving F1 pups were sacrificed after weaning on postnatal day (PND) 21. Clinical chemistry, hematology, and urinalysis parameters were measured in the F0 animals near the end of the study (approximately 12 weeks from initiating exposure), after which the F0 animals were sacrificed and assessed for gross pathology. Key treatment-related findings are also summarized in Table 4-5.

Table 4-5. Selected findings from one-generation reproductive toxicity study in Wistar rats exposed to THF in drinking water

Generation, sex	Parameter[a]	Concentration (ppm) 0	4,000	8,000	12,000
F0 Generation					
Males	THF intake (mg/kg-day)	0	444	795	1,107
Females	THF intake (mg/kg-day): Premating Gestation Lactation All periods	 0 0 0 0	 467 434 714 503	 798 758 1264 890	 1,088 1,139 1,847 1,240
Males	Food consumption (g/day)	28.3 ± 1.81	28.1 ± 1.87	27.0 ± 1.57	25.9 ± 1.79[b]
Females	Food consumption (g/day)	19.9 ± 0.54	20.5 ± 0.72	18.8 ± 0.67	19.6 ± 0.62
Males	Water consumption (g/day)	28.2 ± 1.80	26.8 ± 1.91	23.7 ± 1.60[b]	21.5 ± 1.94[b]
Females	Water consumption (g/day)	21.1 ± 0.92	19.8 ± 1.09	16.2 ± 0.73[b]	15.1 ± 0.87[b]
Males	Body weight (g)	355.4 ± 31.61	356.7 ± 32.09	342.0 ± 46.72	327.0 ± 34.32
Females	Body weight gain (g)	104.6 ± 14.62	115.7 ± 15.75	100.9 ± 9.94	104.4 ± 12.42
Males	Absolute kidney weight (g)	3.071 ± 0.178	3.032 ± 0.223	3.101 ± 0.289	3.141 ± 0.302
Females	Absolute kidney weight (g)	2.012 ± 0.157	2.115 ± 0.202	2.036 ± 0.12	2.153 ± 0.167
Males	Relative kidney weight (%BW)	0.654 ± 0.047	0.647 ± 0.021	0.680 ± 0.036	0.705 ± 0.049[b]
Females	Relative kidney weight (%BW)	0.717 ± 0.034	0.735 ± 0.035	0.775 ± 0.04[b]	0.783 ± 0.048[b]
F1 Generation (pups)					
Males	Body weight gain (g) PND 4–21	44.0 ± 3.16	42.4 ± 3.52	40.6 ± 3.18[b]	37.6 ± 5.33[b]
Females	Body weight gain (g) PND 4–21	42.7 ± 3.50	40.3 ± 2.60	38.0 ± 3.26[b]	36.2 ± 4.44[b]

[a]All values except for THF intake are shown as mean ± standard deviation (SD); THF intake shown as means.
[b]Statistically different ($p \leq 0.05$) from controls.

Source: BASF (1994).

Food consumption was statistically significantly reduced in the high-dose F0 males and in the mid-dose F0 females. Water consumption was statistically significantly decreased in both sexes at the mid- and high-doses. No mortalities were recorded in either the F0 or F1 rats at any exposure concentration. No effects were observed for any measured reproductive endpoint. However, relative kidney weights were statistically significantly increased in high-dose F0 males and in mid- and high-dose F0 females. In the F1 generation, numbers of pups, sex ratio, and viability/mortality were comparable to controls. Mean body weight gains of both male and female F1 pups were statistically significantly decreased in both the mid- and high-dose groups. EPA identified the NOAEL for this study as 571 mg/kg-day and the LOAEL as 1,005 mg/kg-day based on decreased pup body weight gain and using time-weighted average maternal THF intake during gestation and lactation.

The results from this range-finding study were used to select dose levels for a two-generation developmental and reproductive toxicity study of THF administered to rats in drinking water (Hellwig et al., 2002; BASF, 1996). Wistar rats (25/sex/group) received THF in their drinking water at concentrations of 0, 1,000, 3,000, or 9,000 ppm for 70 days prior to mating and throughout cohabitation, gestation, and lactation. THF intake values estimated from measured water consumption and body weights are shown in Table 4-6. Before weaning, 25 F1 pups/sex/group were randomly selected to be the F1 parental animals. The remaining F1 pups were sacrificed. After the F1 generation pups were weaned, the F0 animals were sacrificed. The F1 animals were exposed continuously to THF at the same concentrations as their parents from weaning and throughout cohabitation, gestation, and lactation. THF intake values estimated from measured water consumption and body weights are shown in Table 4-6.

Endpoints evaluated in F0 and F1 parental animals included food and water consumption, body weight, mortality, and clinical signs. In addition, necropsy was performed on all parental animals at sacrifice, and organ weights were obtained for kidney, liver, testes, and epididymis. Histopathology was performed on all gross lesions, liver, kidney, reproductive organs, and GI organs of sacrificed parental animals. Reproductive endpoints evaluated include mating index, fertility index, gestation index, and live birth index. Litter/delivery endpoints for both F1 and F2 generations included total number of pups, number of live and stillborn pups, sex ratio, clinical signs, body weight, viability index, and lactation index. In addition, pups were evaluated for developmental stages (pinna unfolding, opening of auditory canal, opening of eyes) and behavioral tests (grip reflex, acoustic startle, and pupil constriction). Culled pups, surplus pups, and all pups that died before weaning were assessed macroscopically and, if abnormalities were found, were evaluated by skeletal staining and histological processing of the head. Key treatment-related findings are summarized in Table 4-6.

Table 4-6. Selected findings from a two-generation reproductive toxicity study in Wistar rats exposed to THF in drinking water

Sex	Parameter[a]	Concentration (ppm) 0	1,000	3,000	9,000
F0 Generation					
Males	THF intake (mg/kg-day)	0	91	268	714
Females	THF intake (mg/kg-day): Premating Gestation Lactation All periods	 0 0 0 0	 104 104 166 112	 301 288 478 322	 742 790 1,365 835
Males	Food consumption (g/day)	27.3 ± 1.35	27.2 ± 1.39	27.0 ± 1.48	26.5 ± 1.42
Females	Food consumption (g/day): Premating Gestation Lactation	 19.9 ± 0.61 25.0 ± 1.03 47.8 ± 12.34	 20.0 ± 0.79 25.1 ± 1.28 47.4 ± 10.08	 19.6 ± 0.73 24.4 ± 1.26 46.6 ± 10.17	 18.3 ± 0.71[b] 23.4 ± 1.38[b] 46.0 ± 9.46[b]
Males	Water consumption (g/day)	26.9 ± 1.35	25.8 ± 1.12	25.1 ± 1.03	22.0 ± 0.99[d]
Females	Water consumption (g/day): Premating Gestation Lactation	 20.6 ± 1.21 32.2 ± 6.63 57.3 ± 16.06	 19.7 ± 1.15 29.7 ± 6.10 52.4 ± 12.70[b]	 19.1 ± 0.95[b] 27.9 ± 5.75[b] 50.7 ± 12.05[b]	 15.1 ± 1.00[b] 24.3 ± 5.81[b] 45.9 ± 11.27[b]
Males	Body weight (g)	379.3 ± 53.52	378.3 ± 36.69	374.3 ± 40.73	364.2 ± 39.41
Females	Body weight gain (g): Premating Gestation Lactation	 138.3 ± 17.30 129.7 ± 15.46 9.7 ± 14.02	 138.9 ± 16.31 127.0 ± 14.01 3.7 ± 12.10	 141.7 ± 13.59 124.7 ± 21.03 9.9 ± 9.71	 128.5 ± 14.25 128.3 ± 15.82 7.9 ± 9.53
Males	Absolute kidney weight (g)	3.244 ± 0.301	3.203 ± 0.284	3.104 ± 0.272	3.438 ± 0.27[b]
Females	Absolute kidney weight (g)	2.092 ± 0.113	2.126 ± 0.142	2.159 ± 0.146	2.123 ± 0.133
Males	Relative kidney weight (%BW)	0.665 ± 0.052	0.662 ± 0.057	0.641 ± 0.059	0.719 ± 0.059[b]
Females	Relative kidney weight (%BW)	0.749 ± 0.039	0.774 ± 0.05	0.774 ± 0.054	0.785 ± 0.033[b]
F1 Generation (Pups)					
Maternal THF intake (mg/kg-day), TWA of F0 gestation and lactation periods[a]:		0	134	381	1071
Male pups	Body weight gain (g): PND 4–21 PND 1–4 PND 4–7 PND 7–14 PND 14–21	 45.4 ± 3.04 3.0 ± 0.57 6.1 ± 0.57 17.8 ± 1.15 21.4 ± 2.37	 46.3 ± 3.23 3.3 ± 0.96 6.0 ± 0.71 17.5 ± 1.55 22.7 ± 1.80	 44.8 ± 3.63 2.7 ± 0.80 5.8 ± 0.74 17.2 ± 1.43 21.9 ± 2.03	 41.7 ± 3.38[b] 2.6 ± 0.53 5.5 ± 0.75[b] 15.7 ± 1.65[b] 20.5 ± 1.84
Female pups	Body weight gain (g): PND 4–21 PND 1–4 PND 4–7 PND 7–14 PND 14–21	 43.3 ± 2.72 2.8 ± 0.60 5.9 ± 0.50 17.3 ± 1.47 20.1 ± 1.97	 44.0 ± 3.45 3.1 ± 0.85 5.6 ± .10 17.4 ± 1.72 20.7 ± 1.86	 42.3 ± 2.61 2.7 ± 0.80 5.5 ± 0.52 16.9 ± 1.66 19.9 ± 1.42	 40.1 ± 3.46[b] 2.6 ± 0.51 5.3 ± 0.65[b] 15.6 ± 1.56[b] 19.2 ± 1.84

Table 4-6. Selected findings from a two-generation reproductive toxicity study in Wistar rats exposed to THF in drinking water (continued)

Sex	Parameter[a]	Concentration (ppm) 0	1,000	3,000	9,000
F1 Generation					
Males	THF intake (mg/kg-day)	0	98	293	788
Females	THF intake (mg/kg-day): Premating Gestation Lactation All periods	 0 0 0 0	 125 107 152 125	 358 318 455 362	 882 792 1,165 898
Males	Food consumption (mg/kg-day)	28.0 ± 1.90	28.3 ± 1.77	28.1 ± 1.98	26.3 ± 1.99[b]
Females	Food consumption (mg/kg-day) Premating Gestation Lactation	 21.1 ± 0.50 26.6 ± 1.53 47.0 ± 13.63	 21.4 ± 0.44 26.7 ± 1.46 44.8 ± 11.93	 21.0 ± 0.44 26.6 ± 1.33 44.6 ± 12.69	 20.9 ± 0.68 26.0 ± 1.42 40.5 ± 11.55[b]
Males	Water consumption (g/day)	27.9 ± 2.07	29.2 ± 2.23	28.8 ± 2.65	24.2 ± 2.39[b]
Females	Water consumption (g/day): Premating Gestation Lactation	 23.5 ± 1.28 32.3 ± 7.74 57.0 ± 15.32	 25.9 ± 1.63 33.8 ± 8.00 52.5 ± 10.82	 24.0 ± 1.07 33.1 ± 6.54 52.1 ± 11.64	 19.5 ± 0.89[b] 27.7 ± 6.53[b] 43.6 ± 10.93[b]
Males	Body weight (g)	453.4 ± 40.49	456.6 ± 35.27	458.4 ± 53.88	426.1 ± 37.39
Females	Body weight gain (g): Premating Gestation Lactation	 198.0 ± 19.39 127.1 ± 17.23 10.9 ± 13.44	 201.0 ± 22.92 128.0 ± 14.22 4.6 ± 12.86	 204.5 ± 23.68 125.0 ± 19.18 7.6 ± 10.99	 208.0 ± 24.49 112.6 ± 17.79[b] 9.4 ± 14.05
Males	Absolute kidney weight (g)	3.233 ± 0.455	3.208 ± 0.192	3.201 ± 0.348	3.181 ± 0.338
Females	Absolute kidney weight (g)	2.347 ± 0.144	2.364 ± 0.201	2.365 ± 0.2	2.411 ± 0.153
Males	Relative kidney weight (%BW)	0.62 ± 0.099	0.606 ± 0.041	0.608 ± 0.05	0.642 ± 0.058
Females	Relative kidney weight (%BW)	0.805 ± 0.048	0.8 ± 0.066	0.812 ± 0.043	0.826 ± 0.059

Table 4-6. Selected findings from a two-generation reproductive toxicity study in Wistar rats exposed to THF in drinking water (continued)

Sex	Parameter[a]	Concentration (ppm)			
		0	1,000	3,000	9,000
F2 Generation					
Maternal THF intake (mg/kg-day), TWA of F1 gestation and lactation periods[a]:		0	129	385	974
Male pups	Body weight gain (g): PND 4–21 PND 1–4 PND 4–7 PND 7–14 PND 14–21	 42.6 ± 3.55 2.7 ± 0.85 5.7 ± 0.95 17.4 ± 1.56 19.4 ± 2.23	 43.8 ± 4.67 3.0 ± 1.22 5.8 ± 0.82 17.9 ± 1.98 20.2 ± 2.63	 41.5 ± 4.64 2.7 ± 1.00 5.3 ± 1.15 17.0 ± 1.94 19.2 ± 2.07	 39.5 ± 3.13[b] 3.0 ± 0.75 5.0 ± 0.63[b] 15.6 ± 1.67[b] 18.9 ± 1.71
Female pups	Body weight gain (g): PND 4–21 PND 1–4 PND 4–7 PND 7–14 PND 14–21	 40.7 ± 3.67 2.7 ± 0.71 5.6 ± 0.75 17.2 ± 1.50 17.9 ± 2.26	 41.2 ± 3.35 2.7 ± 1.10 5.2 ± 1.32 17.1 ± 1.62 18.6 ± 1.83	 38.7 ± 4.67 2.4 ± 1.11 5.0 ± 1.12 16.0 ± 2.41 17.8 ± 2.69	 38.1 ± 3.67 2.9 ± 0.75 5.0 ± 0.64 15.4 ± 1.84[b] 17.6 ± 2.15
All pups	% with eyes open on PND 15	89.9 ± 22.73	98.7 ± 4.13	94.0 ± 12.43	79.2 ± 31.18[b]

[a]All values except for THF intake are shown as mean ± SD; THF intake shown as mean. TWA = time-weighted average using 22 days for gestation and 21 days for lactation. PND = postnatal day. BW = body weight.
[b]Statistically significantly different ($p \leq 0.05$) from controls using the Dunnett test (2-sided).

Sources: Hellwig et al. (2002); BASF (1996).

In the F0 generation, food consumption of the high-dose females was statistically significantly reduced during selected weekly measurements compared with controls during the premating period, gestation, and lactation. Water consumption for males in the high-dose group was statistically significantly decreased during the premating period, and for the mid- and high-dose females it was statistically significantly decreased during the premating period, gestation, and lactation. In high-dose females, body weights were statistically significantly decreased compared with controls during selected periods during premating, gestation, and throughout lactation, but no significant change in body weight gain was observed. No clinical signs related to THF were observed in either F0 males or females at any dose. In F0 males, the mating index and fertility index were comparable among the controls and treated groups. Similarly, the mating and fertility indices for F0 females were comparable among control and treated groups. The mean duration of gestation was similar in all groups and the gestation index was 100% for all groups. Absolute kidney weight was increased in high-dose males, and relative kidney weight was significantly increased in both high-dose male and female F0 rats. No treatment-related gross lesions or microscopic findings were observed in either males or females.

The total number of F1 pups delivered, the number of live and stillborn pups, and the sex ratio were comparable among the groups. In the low-dose group, nine F1 pups from a single litter died between days 1 and 10. Also, two dams in the mid-dose group cannibalized pups. Therefore, the lactation index for these dose groups is statistically significantly decreased compared to controls. However, the authors concluded that this decrease is not related to administration of THF, because there was no dose-response relationship. The mean body weights and body weight gains of the F1 pups in the high-dose group were significantly decreased during PNDs 4–7 and PNDs 7–14. The treated F1 pups did not demonstrate any clinical signs, changes in developmental stages, changes on behavioral tests, or findings on necropsy compared with controls.

Food consumption was significantly decreased in high-dose F1 male adult rats during the premating period and in high-dose F1 female rats during lactation. Water consumption was significantly decreased in high-dose F1 male rats during the premating period and in the high-dose F1 females during the premating period, gestation, and lactation. In high-dose F1 males, slight but significant decreases in body weight were observed throughout the study, but no effect on body weight gain was observed. No effects on body weight or body weight gain were observed in F1 female adults. No clinical signs related to THF were observed in either F1 males or females at any dose. In F1 males and females, the mating and fertility indices were comparable among the controls and treated groups. The mean duration of gestation was similar in all groups, and the gestation index was 100% for all groups. No treatment-related effects on organ weight, gross lesions, or microscopic findings were observed in either the male or female F1 adult rats at any exposure concentration.

The mean number of delivered F2 pups/litter was decreased 16% in the high-dose group compared with control (12.4, 13.0, 12.9, and 10.4 in the 0, 1,000, 3,000, and 9,000 ppm dose groups, respectively) and was outside the range of historical control values of 11.1–16.4 (Hellwig et al., 2002; BASF, 1996). The study authors concluded that this was a spontaneous finding and was not related to treatment since it was not seen in the F0 generation or in the range-finding study; the decrease was limited to a few litters with ≤6 pups/litter (BASF, 1996). Data on the number of implantations and resorptions were not reported. Also, one F1 parental male rat in the high dose group was found to be infertile which, in the absence of corroborating histopathology findings, was considered a spontaneous finding (BASF, 1996). The number of stillborn pups was statistically significantly increased in the two lower dose groups, but not in the high-dose group. Based on the lack of dose-response relationship, the authors concluded that all of these findings were spontaneous and not related to THF administration. In the low- and mid-dose groups, there was an increase in the number of pups cannibalized or dead before scheduled sacrifice. As a result, the viability index was statistically significantly decreased in the low-dose group; the viability indices for the mid- and high-dose groups were comparable to controls. Body weight gain was statistically significantly reduced in the high-dose male and female F2 pups during PNDs 7–14. A significant number of F2 pups/litter in the high dose group had delayed opening of eyes (% with eyes open on PND 15); 79 compared to 90% in controls, historical control range 85–100%). Also, there was an increase in the number of sloped incisors in the high dose F2 litters (mean 1.5% of pups/litter compared to 0% in controls; historical control range 0–2.9%). The study authors considered this finding to be consistent with a slight developmental delay (Hellwig et al., 2002). The mean percentage of F2 pups/litter with open auditory canal was 96.4, 100, 88.9, and 98.9% in the 0, 1,000, 3,000, and 9,000 ppm dose groups, respectively. This finding was discounted by the study authors because it was not dose-related and the statistically significantly different value of 88.9% in the mid-dose group fell within the historical control range of 81–100%. Values for lactation index, sex ratio, clinical signs, behavioral tests, and necropsy findings were comparable between controls and treated animals.

In the high dose groups, general toxicity was indicated by slight to marginal decreased food consumption, decreased body weight, and increased kidney weight in F0 adults and decreased food consumption and body weight gain in F1 adults. However, decreased adult body weights were only observed during selected periods during the study, were of minimal severity, and were not generally reflected by changes in body weight gain. Therefore, the adult body weight changes were not considered to be of sufficient magnitude to identify an adverse effect level.

No clinical signs (in the one- or two-generation studies) or clinical chemistry changes (only measured in the one-generation study) consistent with dehydration were observed, suggesting that the decrease in water consumption was not inducing changes in maternal health. The study authors stated that the reduced water consumption observed in the mid- and high-dose F0 and high-dose F1 parental rats was most likely due to reduced palatability of the THF in drinking water. The reduction in water intake averaged 7% during premating and 12–14% during gestation and lactation following exposure to 3,000 ppm THF. There were no corresponding decreases in food consumption at this dose during these time periods. Thus, Hellwig et al. (2002) concluded that the reduction in water consumption was biologically insignificant and that the NOAEL for systemic toxicity (increased relative kidney weight, body weight gain, and food consumption) in F0 and F1 parental rats was 3,000 ppm.

Pup body weight gain was reduced at the high dose during PNDs 4–7 and 7–14 in both F1 and F2 pups. This reduction in weight gain may be due to reduced maternal milk production, but the study authors indicated that it was not related to maternal body weight or water consumption. Specifically, maternal body weight was reduced significantly in the F0 dams and not the F1 dams during lactation. Data on the possible relationship between decreased water intake in dams and decreased production of milk was not provided in this study. Hellwig et al. (2002) stated that decreased pup body weight gain could be related to direct exposure to THF during lactation. Specifically, the study authors suggested that given that THF is slightly more soluble in lipid than water, THF may have been more concentrated in the dam's milk fat than in the maternal water compartment. Based on the developmental effects observed (decreased pup body weight gain, delayed eye opening, and increased incidence of sloped incisors) the study authors designated 3,000 ppm as the NOAEL. The finding of decreased mean number of F2 pups delivered/litter in the high-dose group (10.4 versus 12.4 in control) is also supportive.

While the two-generation study demonstrated a decrease in pup body weight gain in both the F1 and F2 generations following THF exposure, the contribution of other potential confounding factors, such as dam water consumption and litter size (which may influence the milk availability to each pup), were considered further using multivariable regression analyses. The regression analyses included pup body weight gain during PNDs 7–14 as the dependent variable and four independent variables: average THF intake, maternal water intake during lactation, number of pups in each litter (during the affected postnatal period), and a categorical variable for the dose group. Since the response data from F1 and F2 generation are independent, these data were analyzed separately. Preliminary regression analyses suggested that there was a high degree of colinearity among the independent variables, as indicated by the high variance inflation factors, and the dose group is the most significantly affected factor. Removal of this factor diminishes the colinearity in the regression. Therefore, in a second series of regression analyses, dose group was not included as an independent variable. The results from this regression analysis are summarized in Table 4-7.

Table 4-7. Correlations between decreased pup body weight gain and each of three independent variables: maternal water intake, THF intake, and number of pups in each litter

	Coefficient	*p*-Value
F1 pup body weight gain (adjusted r^2 = 0.36)		
Average water intake	9.09×10^{-2}	<0.0001[a]
Average THF intake	-3.98×10^{-4}	0.1458
Number of pups	-4.23×10^{-1}	0.0335[a]
F2 pup body weight gain (adjusted r^2 = 0.24)		
Average water intake	5.90×10^{-2}	0.0015[a]
Average THF intake	-8.51×10^{-4}	0.0218[a]
Number of pups	-5.04×10^{-1}	0.0055[a]

[a]Statistically significant correlation at $p < 0.05$.

Source: An EPA analysis based on Hellwig et al. (2002); BASF (1996).

Based on the results from multiple regression analyses, the dependent variable (pup body weight gain) can be predicted from a linear combination of the independent variables of average water intake, THF intake, and number of pups in each litter. For F1 pups, there is no evidence to suggest a statistically significant correlation ($p = 0.1458$) between maternal THF intake and pup body weight gain when controlling the other confounding factors, such as maternal water intake and number of pups in each litter. However, the similar analysis for the F2 pup data indicates that there is a significant correlation ($p = 0.0218$) between pup body weight gain and maternal THF intake after controlling for the other confounding factors. The study authors concluded that the high concentration effects reflect general toxicity of THF, while noting that decreased water (and food) intake could have contributed to the observed decrease in body weights.

Based on the parental findings of decreased body weight and the developmental effects of decreased pup body weight gain, delayed eye opening, and increased incidence of sloped incisors, EPA identified the NOAEL as 3,000 ppm and the LOAEL as 9,000 ppm for this study. The best value to use for estimating the corresponding doses (mg/kg-day) differs for each generation, based on THF intake values over the relevant period of exposure. For parental effects, time-weighted average (TWA) THF intakes over the entire study period are appropriate for use in assigning effect levels. For developmental effects, the TWA THF intake during the gestation and lactation period of the parent females was used to estimate the effective dose. Table 4-8 summarizes the corresponding effect level doses across all endpoints that showed a treatment-related effect.

Table 4-8. Summary of effect levels observed in the two-generation reproduction study in Wistar rats exposed to THF in drinking water

Effect	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)
F0 Males—increased kidney weight	268	714
F0 Females—decreased body weight, increased kidney weight	322	835
F1 Adult males—decreased body weight gain	293	788
F1 Adult females—decreased body weight gain	362	898
F1 Pups—decreased body weight gain	381	1,071
F2 Pups—decreased body weight gain, delayed eye opening and increased incidence of sloped incisors	385	974

Sources: Hellwig et al. (2002); BASF (1996).

4.3.2. Inhalation

Mast et al. (1992) assessed developmental toxicity of THF in mice and rats. Female CD-1 mice (10 virgin and 30 mated animals/group) were exposed to 0, 600, 1,800, or 5,000 ppm (0, 1,770, 5,310, or 14,750 mg/m^3) THF vapor for 6 hours/day, 7 days/week on gestation days 6–17. Female mice in the 5,000 ppm group demonstrated a high toxicity, with >25% mortality observed after only 6 days of exposure. Consequently, mice in this group were removed from exposure at this time and placed in a chamber with fresh air until time of scheduled sacrifice. Developmental evaluations were conducted on pregnant mice euthanized on gestation day 18. Developmental endpoints included gross maternal toxicity and number, position, and status of implantation sites. Live fetuses were weighed, sexed, and examined for gross defects. Half of the live fetuses and any fetus with gross defects were examined for visceral defects, and the heads were examined for soft-tissue craniofacial abnormalities. All fetal carcasses were examined for gross changes in cartilage and ossified bone. Maternal deaths occurred in the high-concentration group. Other statistically significant maternal effects that were observed at concentrations of ≥1,800 ppm included narcosis, decreased terminal body weight, reduced adjusted maternal weight gain (adjusted for uterine weight), and reduced gravid uterine weight. A reduction in the percent live pups/litter and delayed ossification of the sternum were observed at concentrations of ≥1,800 ppm. Surviving pregnant mice in the high concentration group had litters with a 95% resorption incidence; however, if the conceptus survived, development continued normally. There were no effects on the number of implantations, the fetal sex ratio, or the incidence of abnormalities in fetuses. Based on decreased gravid uterine weight in dams and reduced fetal survival, EPA identified the LOAEL as 1,800 ppm (5,310 mg/m^3) and the NOAEL as 600 ppm (1,770 mg/m^3) in mice.

Pregnant Sprague-Dawley rats (10 virgin and 30 mated animals/group) were exposed to 0, 600, 1,800, or 5,000 ppm (0, 17, 70, 5,310, or 14,750 mg/m^3) THF vapor for 6 hours/day, 7 days/week on gestation days 6–19 (Mast et al., 1992). Developmental evaluations were conducted on pregnant rats euthanized on gestation day 20. Developmental endpoints included gross maternal toxicity and the number, position, and status of implantation sites. Live fetuses were weighed, sexed, and examined for gross defects. Half of the live fetuses and any fetus with gross defects were examined for visceral defects, and the heads were examined for soft-tissue craniofacial abnormalities. All fetal carcasses were examined for cartilage and ossified bone. In dams, the cumulative body weights were significantly reduced in the high concentration group throughout the exposure period. In addition, nonsignificant reductions of gravid uterine weight and extragestational weight gain (adjusted for uterine weight) were observed in the high concentration group. Fetal rat weights were significantly reduced at 5,000 ppm. There were no effects on the number of implantations, fetal sex ratio, or incidence of fetal abnormalities. Based on decreased maternal and fetal weight, EPA identified the LOAEL as 5,000 ppm (14,750 mg/m^3) and the NOAEL as 1,800 ppm (5,310 mg/m^3) in rats.

DuPont Haskell Laboratory (1980) investigated the effects of inhaled THF on the developing fetus in rats. The authors first performed a range-finding study in which Crl:CD® rats (7–14/group) were exposed by inhalation (whole body exposure) to THF at nominal concentrations of 0, 200, 500, 2,500 and 5,000 ppm (0, 590, 1,475, 7,375, or 14,750 mg/m^3) 6 hours/day on gestation days 6–15. In a follow-up study, Crl:CD® rats (29/group) were exposed to nominal THF concentrations of 0, 1,000, and 5,000 ppm (0, 2,950, or 14,750 mg/m^3) 6 hours/day on gestation days 6–15. Body weight, clinical signs, and feed consumption were observed in dams during the exposure period. Dams were sacrificed on gestation day 21 and were examined for gross pathologic changes, liver weight, and reproductive status. The number of corpora lutea, implantation sites, and live and dead fetuses were recorded. Live fetuses were weighed, sexed, and examined for external alterations. One-third of all fetuses and all stunted or malformed fetuses were examined for visceral alterations, and the heads were fixed for evaluation of eye malformations. Remaining fetuses were fixed and stained for examination of skeletal alterations. The same endpoints were examined in both parts of the study.

No mortality was observed in dams in either study. In both studies, dams in the high-concentration group demonstrated decreased response to noise stimulus, reduced muscle tone, and staggering gait that persisted for about 1 hour following each daily exposure period. In addition, dams in the lower concentration group (7,375 mg/m^3 in the range-finding study and 2,950 mg/m^3 in the main study) had a diminished response to noise stimulus. Food consumption in the main study high-concentration group was significantly reduced compared to controls. In both studies, dams in the high-concentration group had significantly reduced body weight gain compared to controls. The number of implants/dam and mean fetal body weight both were significantly decreased with increasing exposure (although no information is provided on which concentration-level significance was first observed). In addition, fetuses in the high-concentration group exhibited a significantly decreased incidence of sternal ossification. Based on decreased fetal weight and skeletal alterations, EPA identified the developmental LOAEL as 14,750 mg/m^3 and the NOAEL as 7,375 mg/m^3. Based on clinical signs of sedation (diminished response to noise stimulus), the maternal LOAEL and NOAEL were 2,950 and 1,475 mg/m^3, respectively.

4.4. OTHER DURATION- OR ENDPOINT-SPECIFIC STUDIES

Several short-term oral studies in animals are available (see Appendix C for study descriptions). In rats treated with a total of six gavage doses of THF in distilled water, increased mortality was observed at doses >2,000 mg/kg (Stasenkova and Kochetkova, 1963). Toxicity observed in this study included CNS toxicity (immobility, drowsiness, reduced response to external stimuli) and necrosis, edema, and hemorrhage of stomach, brain, liver, heart, spleen, and kidneys. However, it is not possible to more fully characterize the specific histopathology endpoints in the study by Stasenkova and Kochetkova (1963). In a 4-week study of THF in drinking water administered to rats (Komsta et al., 1988), doses as high as 96 mg/kg-day had no effect on mortality and did not produce clinical signs of CNS toxicity in rats. Histopathologic lesions in liver (increased cytoplasmic homogeneity and anisokaryosis) and kidney (tubular cytoplasmic inclusions) were observed in the high-dose group males and females.

Several acute inhalation studies in animals suggest that the primary effects observed following single exposures to THF—ranging from 30 minutes to several hours—are CNS toxicity and respiratory tract irritation. Symptoms of CNS toxicity, including sedation, coma, altered respiration, and decreased response to external stimuli, were observed in dogs (Stoughton and Robbins, 1936), mice (Stasenkova and Kochetkova, 1963; Stoughton and Robbins, 1936), and rats (Horiguchi et al., 1984; DuPont Haskell Laboratory, 1979; Stasenkova and Kochetkova, 1963). Clinical signs of respiratory tract irritation, observed only in rat studies, included scratching, head shaking, face washing, tearing, salivation, and bleeding from the nose (Horiguchi et al., 1984; DuPont Haskell Laboratory, 1979). In addition, several other acute studies observed structural or functional changes in respiratory tissue (suggesting respiratory tract irritation), including congested mottled lungs in rats (Henderson and Smith, 1936), edema and hemorrhage in lungs and bronchi of rats (Stasenkova and Kochetkova, 1963), and decreased ciliary beat frequency and vacuolation/degeneration of both nasal mucosa (Ohashi et al., 1983) and tracheal mucosa (Ikeoka et al., 1988) in rabbits, and nasal and tracheal histopathology changes in rats (Horiguchi et al., 1984). Two studies report histopathological lesions in other organs such as liver (Stasenkova and Kochetkova, 1963; Henderson and Smith, 1936), kidney, brain, and spleen (Stasenkova and Kochetkova, 1963). However, Hofmann and Oettel (1954) specifically examined the liver and kidney and found no effects. These studies are further described in Appendix C.

4.5. MECHANISTIC DATA AND OTHER STUDIES

Genotoxicity.

Only one study that evaluated genotoxicity endpoints in humans was identified. Funes-Cravioto et al. (1977) reported increased chromosome breaks in peripheral lymphocytes from solvent-exposed versus nonexposed adults. However, of the seven occupational groups that were pooled for the statistical analysis, only one was identified as having used THF in the workplace (no exposure information was provided by the study authors), thus suggesting that agents other than THF likely played a greater role in the observed genotoxicity.

NTP (1998) presented the results of a battery of mutagenicity/genotoxicity tests of THF. The in vitro tests included the *Salmonella typhimurium* bacterial mutagenicity assay (with and without S9 microsomal activation), induction of sister chromatid exchange and chromosomal aberrations in the Chinese hamster ovary cell system, and in vivo in mouse bone marrow cells. Micronuclei frequency in peripheral blood erythrocytes following 14-day inhalation exposure of mice to THF was also evaluated. NTP (1998) concluded that there was little evidence of mutagenic activity, with most data determined to be conclusively negative.

In summary, the genotoxic potential of THF has been evaluated in a variety of in vitro and in vivo assays. Nearly all the results are conclusively negative, with equivocal findings reported in a small number of assays that have been conducted. The genotoxicity data are summarized in Table C-5 and discussed in more detail in Appendix C.2. Taken together, these data support the conclusion that THF is not likely genotoxic.

4.6. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS

A summary and synthesis of the major noncancer effects observed following oral and inhalation exposure to THF are described in the following sections. The modes of action for the noncancer effects are not known; however, mechanistic data relating to the potential modes of action for the noncancer effects are further described in Appendix C.

4.6.1. Oral

No human studies of THF following oral exposure are available and the oral database for animal studies is limited. A summary of the effects observed in the subchronic oral studies is presented in Table 4-9. In a one-generation reproductive toxicity study (BASF, 1994) of THF administered to rats in drinking water, symptoms of general toxicity—including decreased food (males) and water consumption (males and females) and increased kidney weight (males and females)—were observed in parental generation rats administered 8,000 ppm THF (795 mg/kg-day for males and 890 mg/kg-day for females). At this concentration, male and female pups had significantly decreased body weight gain compared with controls. A follow-up two-generation reproductive toxicity study (Hellwig et al., 2002; BASF, 1996) of THF administered to rats in drinking water demonstrated similar results as the one-generation study in the parental generation rats, including decreased food consumption (F0 females, F0/F1 males), decreased water consumption (F0/F1 both sexes), decreased body weight (F0/F1 both sexes), and increased kidney weight (F0 both sexes) at 9,000 ppm THF (714 mg/kg-day for F0 males, 788 mg/kg-day for F1 males, 835 mg/kg-day for F0 females, 898 mg/kg-day for F1 females). At these same concentrations, the F1 and F2 pups had significantly reduced body weight gain compared with controls, and the F2 pups also demonstrated delayed eye opening and increased incidence of sloped incisors compared with controls at the highest dose (see Table 4-6). Histopathology examination on parental rats included liver, kidney, reproductive organs, and digestive tract organs and demonstrated no observed effects. Exposure at the high concentration of THF in drinking water may have a subtle effect on male rat fertility/fecundity based on a 16% decrease in the mean number of delivered F2 pups (not statistically significant but below the range of historical control values) and a finding of one infertile F1 parental male rat in the high dose group. In both studies, no effects were observed on any other reproductive parameters measured.

Although the data indicate that THF induced an increase in kidney weight in rats, the severity of the impact on the kidneys appears to be minimal. This conclusion is supported by several considerations that were summarized under Section 4.3.1. THF exposure had no effect on absolute or relative kidney weight in F1 generation adults. Furthermore, the kidney weight changes that were observed in the F0 generation were not accompanied by gross kidney pathology or clinical chemistry findings consistent with an effect on renal function (in the one-generation study) or by histopathological examination (in the two-generation study). Additionally, evaluation of the overall database for THF including the inhalation studies does not suggest that THF is a potent kidney toxicant. For example, most of the available human case reports have not identified the kidney as a target of THF exposure. Furthermore, in the subchronic and chronic inhalation NTP (1998) studies, changes in kidney weight or pathology were not particularly sensitive to THF exposure.

Table 4-9. Summary of effects observed in drinking water toxicity studies with THF[a]

Study	Species, number, sex	Route, duration, doses	Observed effects	NOAEL (mg/kg-day)[b]	LOAEL (mg/kg-day)[b]	Comments
BASF (1996); Hellwig et al. (2002)	Wistar rat 25/sex/dose	Drinking water, two-generation reproductive 0, 1,000, 3,000, 9,000 ppm	General toxicity (decreased BW and increased kidney weight in F0 and F1 adults, decreased BW gain in F1/F2 pups, delayed eye opening and increased incidence in sloped incisors in F2 pups	F0 males: 268 F0 females: 322 F1 adult males: 268 F1 adult females: 362 F1 pups: 381 F2 pups: 385	F0 males: 714 F0 females: 835 F1 adult males: 788 F1 adult females: 898 F1 pups: 1,071 F2 pups: 974	Each generation treated 70 days prior to mating through cohabitation, gestation, lactation
BASF (1994)	Wistar rat 10/sex/dose	Drinking water, one-generation reproductive 0, 4,000, 8,000, 12,000 ppm	Increased kidney weight (F0 males—high dose, F0 females—mid dose) Decreased pup BW (mid dose)	503 546	890 960	

[a]The best value to use for estimating the corresponding doses (mg/kg-day) differs for each generation based on THF intake values over the relevant period of exposure. For parental effects, average THF intakes over the entire study period are appropriate for use in assigning effect levels. For developmental effects, the time-weighted average THF intake during the gestation and lactation periods of the parent females was used to estimate the effective dose. THF intake estimates are shown in Table 4-5.

[b]THF intake estimates corresponding to NOAEL and LOAEL estimates were calculated for a variety of effects and are presented in Table 4-8.

Additionally, several effects were noted in short-term studies, (Komsta et al., 1988; Stasenkova and Kochetkova, 1963; described in Appendix C). Increased mortality and effects including CNS toxicity (immobility, drowsiness, reduced response to external stimuli), and necrosis, edema, and hemorrhage of stomach, brain, liver, heart, spleen, and kidneys were observed in rats administered THF in distilled water via gavage (Stasenkova and Kochetkova, 1963). Another short-term study of lower doses of THF administered to rats in drinking water (Komsta et al., 1988), had no effect on mortality and did not produce clinical signs of CNS toxicity. Histopathologic lesions in liver (increased cytoplasmic homogeneity and anisokaryosis) and kidney (tubular cytoplasmic inclusions) were observed in the male and female rats.

4.6.2. Inhalation

Although no epidemiological studies of THF have been conducted, several case studies in humans illustrate the potential for health effects following inhalation exposure in an occupational setting. In almost all of the cases, workers were exposed to THF through activities where THF was present as a component of solvents or adhesives. In general, workers were exposed for a period of a few weeks to a few months before symptoms were reported. Target organs in humans appear to be the CNS, respiratory tract, liver, and kidney. Symptoms of CNS toxicity included headache, dizziness, fatigue, loss of the sense of smell (Garnier et al., 1989; Emmett, 1976; Horiuchi et al., 1967), and convulsions (following enfluran anesthesia in a worker exposed to THF in the weeks prior to surgery) (Juntunen et al., 1984). Symptoms of respiratory tract irritation included cough, chest pain, rhinorrhea, and dyspnea (Garnier et al., 1989; Emmett, 1976). In three cases, liver enzymes (ALT, AST, and GGT) were elevated above normal values (Garnier et al., 1989; Edling, 1982; Horiuchi et al., 1967), and in one case a liver biopsy revealed fatty changes following THF exposure (Edling, 1982). In one study, hematological changes and decreased white blood cell counts were reported in THF-exposed workers (Horiuchi et al., 1967). In one case study, autoimmune glomerulonephritis was observed in a man who worked with THF in adhesives for 9 years (Albrecht et al., 1987). The human case studies suggest that CNS toxicity, respiratory tract irritation, and liver and kidney toxicity are the potential health effects following inhalation exposure to THF.

An uncertainty associated with all of the reported human case studies is the fact that workers were exposed to other solvents and chemicals in addition to THF, so it is not possible to conclusively attribute the observed effects to THF exposure alone. In addition, in most cases quantitative estimates of exposure were not provided.

In animals, subchronic and chronic studies reported several systemic effects following inhalation exposure to THF; a summary of these effects is presented in Table 4-10. Decreased body weight has been observed in rats (Horiguchi et al., 1984; Kawata and Ito, 1984). Decreased blood pressure was observed in dogs (BASF, 1938) and rats (Stasenkova and Kochetkova, 1963). Altered hematological parameters were observed in rats (NTP, 1998; Horiguchi et al., 1984), mice (NTP, 1998; Stasenkova and Kochetkova, 1963), and dogs (BASF, 1938). Following 14 weeks of inhalation exposure, rats of both sexes had significantly increased relative and absolute liver weight (NTP, 1998). In the same study, mice of both sexes showed increased relative and absolute liver weight (NTP, 1998). In addition, Horiguchi et al. (1984) observed increased relative weights of liver. Changes in mice included liver centrilobular cytomegaly in both sexes following 14 weeks of exposure to THF (NTP, 1998). Increased incidence of hepatocellular necrosis was also observed in female mice in the 2-year inhalation study (NTP, 1998).

Inhalation exposure to THF appears to also result in symptoms of CNS toxicity and respiratory tract irritation. In a subchronic neurotoxicity assay (DuPont Haskell Laboratory, 1996b) the only effects observed were transient symptoms of CNS toxicity that were not observed on mornings prior to the start of the weekly exposures. No permanent neurotoxic effects were observed on motor activity or in any functional observational battery (FOB) parameter. Altered brain catecholamine levels were observed following 8 weeks of inhalation exposure (Kawata et al., 1986), and altered EEGs were observed following i.p. injection (Marcus et al., 1976). While the clinical significance of these findings is uncertain in terms of assigning adverse effect levels for THF, the observation that similar brain alterations are induced by the THF metabolites GBL and GHB (NSF, 2003) suggests that these metabolites may be responsible for the observed neurotoxicity of THF.

In two subchronic studies, authors specifically note that symptoms of CNS toxicity (NTP, 1998; Horiguchi et al., 1984) appeared to moderate with continued exposures. Based on findings in Elovaara et al. (1984) of decreased concentrations of THF in rat brain and fat tissues with extended exposure, the authors of the NTP (1998) study considered it likely that the apparent tolerance to the CNS effects may be due to stimulation by THF of its own metabolism. They also concluded that it is not possible to ascertain whether the clinical findings of CNS toxicity (narcosis) were primary (i.e., specific to THF or its metabolites) or secondary (i.e., nonspecific due to solvent interaction with cell membranes of the nervous system as seen with other solvents) and that further research is needed to better characterize THF neurotoxicity.

However, support for the THF-induced CNS effects was provided by evidence of these effects in the subchronic and chronic studies as well as short-term and acute studies. Several acute inhalation studies in animals suggest that one of the primary effects observed following single exposures to THF is CNS toxicity (including sedation, coma, altered respiration, and decreased response to external stimuli in dogs [Stoughton and Robbins, 1936], mice [Stasenkova and Kochetkova, 1963; Stoughton and Robbins, 1936], and rats [Horiguchi et al., 1984; DuPont Haskell Laboratory, 1979; Stasenkova and Kochetkova, 1963]).

Additional effects observed include respiratory tract irritation, kidney effects, thymus weight changes, and effects associated with immunotoxicity and developmental toxicity. Histopathological changes in the respiratory tract were observed in one subchronic study at 3,000 ppm (Kawata and Ito, 1984) and in several acute and short-term studies (Ikeoka et al., 1988; Horiguchi et al., 1984; Ohashi et al., 1983; Stasenkova and Kochetkova, 1963) with some at relatively low exposure concentrations. Specifically, Horiguchi et al. (1984) found nasal histopathology after a 3-week exposure to 100 or 5,000 ppm THF but no such effects were reported following exposure to 5,000 ppm for 12 weeks. Changes in the tracheal mucosa in the group exposed to 5,000 ppm were described as occurring in the cilia, with disorder of the epithelial architecture and darkening of cell bodies. However, the study authors did not clarify whether the nasal effects at 100 ppm were the same as the tracheal effects at 100 or 5,000 ppm, although it was presumed that it was the tracheal effects at 5,000 ppm that were being equated to the 100 ppm nasal effects. The authors did not describe any results for the tracheal mucosa at 100 ppm. A major deficiency in this study is that the results represent a single animal per exposure level at each time point. Based on the small sample size, duration of exposure, absence of clear documentation of the severity of the nasal histopathology, and uncertainty regarding the concentration at which nasal changes were observed, this study provided equivocal results regarding respiratory toxicity. Also, symptoms of eye and respiratory tract irritation as well as changes in the threshold of neuromuscular irritability were found in rats and mice following 2 months of exposure (6,000–8,000 mg/m^3), but similar symptoms were not reported following 6 months of exposure to lower concentrations (1,000–2,000 mg/m^3) (Stasenkova and Kochetkova, 1963). These data demonstrate that the irritation effects induced by THF were not consistently observed with increasing duration of exposure. These effects were observed at higher exposure concentrations than those where liver effects were observed. In addition, there are limitations in documentation and reporting.

Clinical signs of respiratory tract irritation, observed in studies in rats only, included scratching, head shaking, face washing, tearing, salivation, and bleeding from the nose (Horiguchi et al., 1984; DuPont Haskell Laboratory, 1979). In addition, several other acute studies observed structural or functional changes in respiratory tissue (suggesting respiratory tract irritation), including congested mottled lungs in rats (Henderson and Smith, 1936), edema and hemorrhage in lungs and bronchi of rats (Stasenkova and Kochetkova, 1963), and decreased ciliary beat frequency and vacuolation/degeneration of both nasal mucosa (Ohashi et al., 1983) and tracheal mucosa (Ikeoka et al., 1988) in rabbits, and nasal and tracheal histopathology changes in rats (Horiguchi et al., 1984). Two studies report histopathological lesions in other organs such as liver (Stasenkova and Kochetkova, 1963; Henderson and Smith, 1936), kidney, brain, and spleen (Stasenkova and Kochetkova, 1963). However, Hofmann and Oettel (1954) specifically examined the liver and kidney and found no effects.

As reported by NTP (1998), absolute and relative thymus weights were statistically significantly decreased, beginning at 1,770 mg/m^3 in male mice. The thymus weight changes were not accompanied by histopathological changes in the subchronic study. The study authors indicated that the significance of the thymus weight changes was unclear and suggested that these changes might have been due to stress associated with THF administration. However, the thymus weight changes were concentration-dependent, suggesting that if they were stress related, this response would have been secondary to the effects of THF. Organ weights were not reported for the chronic study, and therefore, it is not possible to determine if thymus weight is similarly affected by long-term exposure. Histopathological analysis of the thymus in the chronic study revealed an increase in the incidence of thymic atrophy that was statistically significant in the 5,310 mg/m^3 exposure group. This finding was attributed by the authors to be a secondary response, based on the high incidence of urogenital inflammation observed in the males of the high-concentration group. However, since the increase in infections occurred in the same group that had thymic changes, it cannot be determined whether the thymus weight and histopathology effects increased susceptibility to infection or the inflammation had a stress-related effect on the thymus.

It is unclear whether the observed effects on the thymus in the subchronic and chronic studies (NTP, 1998) represent a functional effect on the immune system, and no data are available to differentiate between mechanisms involving a generalized stress response versus other mechanisms directly targeting the immune system. Evaluation of the THF database as a whole provides inconsistent results related to immune effects, with some studies identifying effects and others showing no effect. Nevertheless, some of the available studies show evidence for potential immunotoxicity. For example, decreased white blood cell counts were reported in a study of workers (Horiuchi et al., 1967) and changes in white blood cell counts were reported in an oral drinking water study (Pozdnyakova, 1965) and in a subchronic inhalation study (Horiguchi et al., 1984). Both thymus and spleen weights were reduced in male and female rats in the subchronic NTP (1998) study. In addition, data for THF metabolites are consistent with potential immunotoxicity. For example, thymic depletion was reported at 262 mg/kg-day GBL in mice in a gavage study (NTP, 1992), although this may have been secondary to an inflammatory response or a factor leading to the susceptibility to inflammation. Toxicokinetic information also provides a possible connection between THF exposure and immune effects, in which the tissue distribution study by Kawata and Ito (1984) reported that the thymus and spleen had significantly higher THF concentrations than other tissues following inhalation exposure to 3,000 ppm THF for 12 weeks.

The predictivity of thymus weight changes for functional immune responses has been studied by Luster et al. (1992) who determined the ability of a variety of common measures of immune toxicity, including thymus/body weight ratios, to predict the immunotoxicity of a series of test compounds in mice. When evaluated as a single measure, thymus/body weight ratios were characterized as an unreliable indicator of immunotoxicity (68% concordance—the ability to correctly identify compounds of known immunotoxic potential). However, thymus/body weight ratio was part of several testing configurations that showed 100% concordance with immunotoxicity, suggesting that this measure can contribute to the immunotoxicity assessment. In addition, the authors noted that the lack of concordance for most assays was generally due to a decreased sensitivity (i.e., failure to detect positive immunotoxicants) not a decrease in specificity (i.e., the ability to correctly identify negative compounds). This suggests that thymus/body weight ratios might underreport immunotoxicity. In a follow-up publication by Luster et al. (1993), a good correlation was reported between immune function assays and changes in host resistance (e.g., increased susceptibility to infection from a challenge agent), although the predictivity of individual assays varied (the concordance was 76% for thymus/body weight ratios).

In summary, there are no studies of host resistance or data from other types of immunotoxicity studies following inhalation exposure to THF. Also, it is unclear whether the observed thymus weight changes had a functional impact on the immune system of mice in the subchronic study (NTP, 1998). For this reason, the biological significance of the decrease in thymus weight is questionable. An area of uncertainty exists for the potential effects of THF on the immune system, specifically with regard to decreased thymus weight.

Developmental studies by the inhalation route have been conducted in both rats (Mast et al., 1992; DuPont Haskell Laboratory, 1980) and mice (Mast et al., 1992). In both studies and both species, maternal toxicity included significant decreases in body weight accompanied by decreases in gravid uterine weight (Mast et al., 1992) or food consumption (DuPont Haskell Laboratory, 1980). Decreased fetal weight was observed at the same concentration that resulted in maternal toxicity in rats (Mast et al., 1992). In both mice (Mast et al., 1992) and rats (DuPont Haskell Laboratory, 1980), decreased fetal survival also occurred at the same concentrations that resulted in maternal toxicity. However, as noted in Section 3.1.1.4 of the EPA *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991a), developmental effects occurring at the same exposure levels as maternal toxicity are still considered to represent developmental toxicity and should not be discounted as being secondary to maternal toxicity. With regard to potential teratogenic effects, Mast et al. (1992) noted that in mice that survived the exposure period, no increase was observed in the incidence of fetal abnormalities. However, an increased incidence of incomplete sternal ossification in rat fetuses was observed (DuPont Haskell Laboratory, 1980).

Table 4-10. Summary of findings in developmental, subchronic, and chronic inhalation studies with THF

Study	Species, sex, number, concentration (mg/m^3)	Duration	Observed effects	NOAEL/LOAEL[a] (mg/m^3)	Comments
Developmental toxicity studies					
Mast et al. (1992)	CD-1 mice, female (40/group) 0, 1,770, 5,310, 14,750	6 hours/day, 7 days/week, gestation days 6–17	Decreased dam body weight and gravid uterine weight, decreased fetal survival	1,770/5,310	
	Sprague-Dawley rat, female (40/group) 0, 1,770, 5,310, 14,750	6 hours/day, 7 days/week, gestation days 6–19	Decreased dam body weight, decreased fetal body weight	5,310/14,750	
DuPont Haskell Laboratory (1980)	Crl:CD BR rat, female (29/group) 0, 590, 1,475, 2,950, 7,375, 14,750	6 hours/day, 7 days/week, gestation days 6–15	Dams: CNS clinical signs Fetal: decreased fetal weight, skeletal alterations	Dams: 1,475/2,950 Fetal: 7,375/14,750	
Subchronic studies					
BASF (1938)	Dog, sex and strain not specified (four/group)	590 mg/m^3: 6 hours/day, 5 days/week, 9 weeks then 1,080 mg/m^3: 6 hours/day, 5 days/week, 3 weeks	Decreased blood pressure	NA[b]/590	No microscopic pathology noted in heart, lungs, spleen, pancreas, or kidneys
Horiguchi et al. (1984)	Sprague-Dawley rat, male (11–12/group) 0, 295, 590, 2,950, 14,750	4 hours/day, 5 days/week, 12 weeks	Body and organ weight changes, altered serum chemistry	2,950/14,750	

Table 4-10. Summary of findings in developmental, subchronic, and chronic inhalation studies with THF

Study	Species, sex, number, concentration (mg/m^3)	Duration	Observed effects	NOAEL/LOAEL[a] (mg/m^3)	Comments
Kawata and Ito (1984)	Wistar rat, male (25/group) 0, 8,850	1 hour/day, 5 days/week, 12 weeks	Decreased body weight, papillary hyperplasia in lung and bronchial epithelium, protein casts/hyaline in kidney	NA/8,850	No information given on incidence of histopathologic lesions or statistical significance
DuPont Haskell Laboratory (1996b); Malley et al. (2001)	Crl:CD BR rat (12–18/sex/group) 0, 1,475, 4,425, 8,850	6 hours/day, 5 days/week, 13–14 weeks	CNS clinical signs	1,475/4,425	This was a subchronic neurotoxicity study. No other neurotoxic effects were observed (i.e., FOB, motor activity, or neuropathology)
NTP (1998)	F344/N rat (10/sex/group) 0, 195, 590, 1,770, 5,310, 14,750	6 hours/day, 5 days/week, 90 days	CNS clinical signs, organ weight changes, hematological effects	5,310/14,750	
	B6C3F$_1$ mouse (10/sex/group) 0, 195, 590, 1,770, 5,310, 14,750	6 hours/day, 5 days/week, 90 days	CNS clinical signs, increased liver weight	1,770/5,310	Decreased thymus weight at lower concentrations and histopathology of the liver, uterus, adrenal gland only at the high concentration
Stasenkova and Kochetkova (1963)	Rat, male, strain not specified (20/group) 1,000–2,000	4 hours/day, 7 days/week, 6 months	Decreased blood pressure, increased leukocyte count, hypertrophy of muscle fibers in bronchi walls and spleen	NA/NA	Air concentration reported as a range; study judged as not suitable for dose-response assessment
Chronic studies					
NTP (1998)	F344/N rat (50/sex/group) 0, 590, 1,770, 5,310	6 hours/day, 5 days/week, 2 years	No noncancer effects observed	5,310/NA	

Table 4-10. Summary of findings in developmental, subchronic, and chronic inhalation studies with THF

Study	Species, sex, number, concentration (mg/m^3)	Duration	Observed effects	NOAEL/LOAEL[a] (mg/m^3)	Comments
	$B6C3F_1$ mouse (50/sex/group) 0, 590, 1,770, 5,310	6 hours/day, 5 days/week, 2 years	CNS clinical signs (males); increased liver necrosis (females)	1,770/5,310	Decreased survival, urogenital tract inflammation and histopathology lesions in bone marrow, lymph nodes, spleen, thymus attributed to infection secondary to observed narcosis

[a]NOAEL/LOAEL from the study concentrations.
[b]NA indicates that the NOAEL or LOAEL was not identified.

4.7. EVALUATION OF CARCINOGENICITY

4.7.1. Summary of Overall Weight of Evidence

Under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), the database for THF provides "suggestive evidence of carcinogenic potential." No human data are available to assess the carcinogenic potential of THF. A 2-year NTP (1998) inhalation cancer bioassay reported an increased incidence of renal tubule adenomas and carcinomas in male F344/N rats (statistically significant exposure-response trend) and an increased incidence of hepatocellular adenomas and carcinomas in female $B6C3F_1$ mice (statistically significant trend). No other treatment-related increases in tumor incidence were observed. NTP (1998) concluded that the data provided *some evidence* for THF carcinogenicity in male rats (renal tubular adenomas and carcinomas) and *clear evidence* of carcinogenicity in female mice (hepatocellular adenomas and carcinomas). There was no evidence of carcinogenic activity in female rats or male mice reported by NTP (1998).

There are some data suggesting that the observed renal tumors in the male rats may be secondary to α_{2u}-globulin accumulation; however, a review of the data available for THF indicates that the data do not support an α_{2u}-globulin-related mode of action (MOA) (Section 4.7.3.1). Another consideration regarding the renal tumors is the possibility that advanced chronic progressive nephropathy (CPN), a spontaneous age-related renal disease of laboratory rodents, may play a role in the incidence of atypical tubule hyperplasia (ATH) and perhaps the THF-induced kidney tumors in male rat kidneys. The NTP 2-year carcinogenicity study on THF (1998) reported no difference in the incidence or severity of CPN in the control versus treated male rats. Although THF did not exacerbate development of CPN, it is possible that it may have exacerbated the development of proliferative lesions within CPN-affected tissue; however, there is no direct evidence in support of this. Thus, the kidney tumors observed in male rats are considered relevant to the assessment of the carcinogenic potential of THF to humans.

For the liver tumors in mice, some mechanistic data suggest that THF may induce cell proliferation and lead to a promotion in the growth of pre-initiated cells. However, key precursor events linked to observed cell proliferation have not been identified and the available data are insufficient to establish a mode of action for the THF liver tumor induction (Section 4.7.3.2). Thus, the liver tumors observed in female mice are considered relevant to the assessment of the carcinogenic potential of THF to humans.

U.S. EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) indicate that for tumors occurring at a site other than the initial point of contact, the weight of evidence for carcinogenic potential may apply to all routes of exposure that have not been adequately tested at sufficient doses. An exception occurs when there is convincing toxicokinetic data that absorption does not occur by other routes. Information available on the carcinogenic effects of

THF via the inhalation route demonstrates that tumors occur in tissues remote from the site of absorption. Information on the carcinogenic effects of THF via the oral and dermal routes in humans or animals is not available. Based on the observance of systemic tumors following inhalation exposure, and in the absence of information to indicate otherwise, it is assumed that an internal dose will be achieved regardless of the route of exposure. Therefore, there is "suggestive evidence of carcinogenic potential" following exposure to THF by all routes of exposure.

4.7.2. Synthesis of Human, Animal, and Other Supporting Evidence

As discussed in Section 4.1, there are no human studies investigating the carcinogenic effects of THF following inhalation exposure. However, the NTP (1998) chronic inhalation exposure bioassay in laboratory animals was adequately designed to assess the carcinogenic potential of lifetime inhalation exposure to THF. This study involved exposure of F344/N rats (50/sex/group) and $B6C3F_1$ mice (50/sex/group) to 0, 200, 600, and 1,800 ppm (0, 590, 1,770, and 5,310 mg/m^3) THF for 6 hours/day, 5 days/week for 105 weeks. For the male rats, a statistically significant treatment-related trend was observed for combined incidences of renal tubular epithelial adenomas or carcinomas (1/50, 1/50, 4/50, and 5/50) (NTP, 1998). The response was predominantly benign except for two carcinomas present at the high exposure concentration. The individual incidences of the kidney adenomas or carcinomas in the high exposure male rats exceeded the incidence of these tumors in F344/N historical controls (rate: 0.9 ± 1.3%; range: 0–4%) but were not statistically significant when compared with the concurrent controls (NTP, 1998).

In female mice, there was a statistically significant increase in incidences of hepatocellular adenomas or carcinomas at the high concentration (1,800 ppm) and a positive trend for these hepatocellular neoplasms across exposure to 200, 600, and 1,800 ppm THF compared with controls (17/50, 24/50, 26/50, and 41/48) (NTP, 1998). The females also showed a statistically significant positive trend in hepatocellular carcinomas (albeit not a significantly increased incidence; 6/50, 10/50, 10/50, and 16/48). There was no significant increase in incidences of hepatocellular adenomas or carcinomas in male mice (35/50, 31/50, 30/50, and 18/50), even after adjustment for differential survival.

A 2-year cancer bioassay by the oral route has been conducted for the THF metabolite GBL (NTP, 1992), which showed no evidence of carcinogenicity in rats (male and female) or female mice, although an increased incidence of adrenal medulla pheochromocytomas and hyperplasia were observed. The authors concluded that there was equivocal evidence of carcinogenic potential. Mechanistic studies for THF following exposure by the inhalation route also suggest that THF itself rather than a metabolite might be responsible for the observed liver and kidney responses. Based on mode of action data and the difference in tumor responses for THF and GBL in NTP (1998, 1992) bioassays, EPA concluded that the cancer bioassay data for THF metabolites were not relevant for the assessment of THF carcinogenicity in humans.

As discussed in Section 4.5, results from genotoxicity and mutagenicity studies for THF are mostly negative and provide very limited evidence to suggest a mutagenic mode of action. All bacterial mutation assays were negative for THF. In vitro genotoxicity assays with eukaryotic cells were also negative with the exception of a slight increase in chromosomal aberrations in Chinese hamster ovary cells with metabolic activation (Galloway et al., 1987). In vivo studies suggest that THF is not likely to be mutagenic; however, studies have not been conducted in target tissues.

4.7.3. Mode of Action Information

Both renal and hepatocellular adenomas and carcinomas are observed following inhalation exposure to THF (NTP, 1998). There are mechanistic data suggesting that the induction of kidney tumors in male rats and liver tumors in female mice may involve the accumulation of α_{2u}-globulin in the kidney and increased cell proliferation in the liver, respectively. However, an analysis of the data as outlined below indicates that there is insufficient evidence to establish the roles of α_{2u}-globulin in THF-induced kidney tumors or cell proliferation in THF-induced liver tumors. THF is not likely to be genotoxic, as the results of the genotoxicity and mutagenicity assays provide little evidence of genotoxic or mutagenic activity, with most data determined to be conclusively negative (Section 4.5). Therefore, the mode of carcinogenic action of THF has not been established.

4.7.3.1. *Kidney Tumors*

Description of the hypothesized mode of action.

Generally, kidney tumors observed in cancer bioassays in laboratory animals are assumed to be relevant to humans. However, a number of chemicals have been shown to induce renal tumors as a result of accumulation of α_{2u}-globulin in hyaline droplets. This accumulation initiates a sequence of events that leads to renal nephropathy and, eventually, renal tubular tumor formation. The phenomenon is unique to the male rat since female rats and other laboratory mammals administered the same chemicals do not accumulate α_{2u}-globulin in the kidney and do not subsequently develop renal tubule tumors (Doi et al., 2007; U.S. EPA, 1991b). Some experimental data suggest that the development of kidney tumors in male rats following exposure to THF may involve an α_{2u}-globulin-mediated mode of action; therefore, an analysis of the data is outlined in the following sections.

Identification of key events.

For chemicals inducing kidney tumors in male rats involving the α_{2u}-globulin accumulation mode of action, the following events occur after binding of the chemicals or their metabolites specifically, but reversibly, to α_{2u}-globulin:

- Increased number and size of hyaline droplets in renal proximal tubule cells of treated male rats;
- Accumulation of hyaline droplets containing α_{2u}-globulin in renal proximal tubules due to the resistance of the α_{2u}-globulin chemical complex to hydrolytic degradation by lysosomal enzymes; and
- Induction of typical pathological lesions associated with α_{2u}-globulin nephropathy (e.g., single-cell necrosis, exfoliation of epithelial cells into the proximal tubular lumen, formation of granular casts, linear mineralization of papillary tubules, and tubule hyperplasia).

Experimental support for the hypothesized mode of action.

Strength, consistency, specificity of association.

Chhabra et al. (1998) published a summary of the NTP (1998) bioassay and presented data on the accumulation of α_{2u}-globulin (as indicated by protein droplets) in the male rat kidney following 13 weeks of exposure to 1,800 ppm THF. The NTP study reported qualitative differences in the appearance of protein droplets of the kidneys of control versus male rats exposed to 1,800 ppm THF. Differences in the appearance and location of protein droplets in the male rat kidneys for control and high-concentration exposed rats were noted. Protein droplets were described as finer and more densely and diffusely distributed in tubular epithelial cells in the outer cortex for control rats. In the high-concentration exposed rats, protein droplets were characterized as coarser and concentrated in scattered foci in the outer cortex. However, the average severity grades for the accumulation of protein droplets did not differ and no other differences in the incidence of non-neoplastic lesions in the male rat kidneys were observed. Therefore, no clear signs of treatment-related pathological lesions in the kidney were found in the NTP (1998) study (Chhabra et al., 1998).

BASF (1998) re-evaluated kidney tissues of male rats to examine the relationship between cell proliferation responses and an increase in kidney tumors following THF administration in the NTP (1998) study. Histopathological examination and evaluation of cell proliferation as measured by proliferating cell nuclear antigen (PCNA) staining was conducted for tissue samples from the 0, 200, 600, and 1,800 ppm (0, 590, 1,770, and 5,310 mg/m^3) exposure groups (10/group) from the NTP (1998) 13 week, subchronic study. Kidney tissues from the cortex, outer stripe of the outer medulla, inner stripe of the outer medulla, and the inner medulla were evaluated separately. The histopathological examination revealed an increased incidence of moderate grade hyaline droplet accumulation in the male rat kidney tissues of the high-concentration exposure group as compared with controls, but these changes were not accompanied by evidence of cell degeneration. No increase in cell proliferation was found in any of the individual kidney compartments or in evaluation of all compartments combined. Cell proliferation index was statistically significantly decreased in individual kidney compartments, although these changes did not show a concentration-dependent pattern. No other differences among controls and exposure groups were noted.

Gamer et al. (2002; BASF, 2001a) conducted a series of mode of action studies for kidney effects in male F344 rats (6/group) at similar THF-exposure concentrations to those that were used in the NTP (1998) cancer bioassay. Endpoints, including α_{2u}-globulin accumulation, cell proliferation, and apoptosis, were evaluated. Animals were placed in one of three groups that were exposed 6 hours/day for either 5 consecutive days, 5 consecutive days followed by a 21-day observation period, or 20 consecutive days over a period of approximately 28 days. Test animals were exposed "nose-only" to average THF concentrations of 0, 598, 1,811, or 5,382 mg/m^3 (0, 199, 604, or 1,794 ppm), corresponding to the concentrations used in the NTP (1998) cancer bioassay. For the animals in each of the four concentration groups, a full necropsy was done, including histopathological evaluation of the kidney. Additional evaluations in these same organs included measurements of cell proliferation (S-phase response by 5-bromo-2-deoxyuridine [BrdU] staining) and terminal deoxynucleotidyl dUTP nick-end-labeling staining (TUNEL) apoptosis assay.

Results of the study (Gamer et al., 2002; BASF, 2001a) provide some evidence for α_{2u}-globulin accumulation. Specifically, THF exposure induced α_{2u}-globulin accumulation in male rats in an exposure-related manner (see Table C-3) after 5- or 20-day exposures (6 hours/day). The accumulation of α_{2u}-globulin as measured by immunohistochemistry was supported by histopathological evaluation of hyaline droplets in the kidneys of control and high-concentration animals exposed to THF for 20 days. The incidence of proximal tubule cells with grade 2 (slightly increased) staining for hyaline droplets (putatively α_{2u}-globulin) was 5/6 for exposed animals versus 1/6 for controls. The study also showed that focal areas of α_{2u}-globulin accumulation corresponded to areas of increased cell proliferation. Although no significant increase in labeling index in the renal cortex was determined by standard assessment methods, focal areas of increased BrdU labeling were noted. Quantitation of these areas revealed increased cell proliferation in subcapsular proximal tubules (cortex 1) in animals exposed to THF at the mid and high concentrations for 20 days and at the high concentration for 5 consecutive days. No increase in labeling was observed in the groups given a 21-day recovery period. An increase in cell proliferation was also noted in the proximal tubules between the outer stripe of the outer medulla and the subcapsular layer (cortex 2) at the highest concentration following 20 exposures. The number of cells undergoing apoptosis was significantly increased in the high-concentration groups exposed for 5 days and observed for 21 days or after 20 exposure days. Marginal increases were observed in the mid-concentration groups for these two exposure regimens, but the results were not statistically significant (see Table C-3).

Kawata and Ito (1984) reported protein casts and hyaline droplets in the kidneys of THF-exposed male Wistar rats. No other details of the study are available.

Dose-response concordance.

THF exposure induced α_{2u}-globulin accumulation in male rats treated under all three exposure regimens in the study by Gamer and coworkers (Gamer et al., 2002; BASF, 2001a). Increases were generally concentration related, with increases at the high concentration ranging from 175 to 280% of control levels for cortex 1 and from 188 to 324% of control levels for cortex 2 among the three exposure regimens. When the whole cortex was used as the labeled area for the analysis, accumulation was significantly elevated beginning at the low concentration and following 5 consecutive days or 20 days of exposure. Maximum effects observed at the high concentration ranged from 178 to 299% of controls among the three exposure regimens. Increased cell proliferation and apoptosis in kidneys of animals exposed to THF for 20 days also appeared to show a dose-response relationship (see Table C-3).

Temporal relationship.

The mode of action data were obtained from short-term exposures (5 or 20 days) of THF. Except for some qualitative differences in the appearance of protein droplets of the kidneys of control versus male rats exposed to 1,800 ppm THF, no signs of treatment-related pathological lesions in the kidney were found in the 2-year bioassay of NTP (1998). No increase in cell proliferation was found in any of the kidney compartments in the 13-week study of BASF (1998). Therefore, a temporal relationship of the key events to male rat kidney tumor induction cannot be established.

Biological plausibility and coherence.

The concordance between α_{2u}-globulin accumulation, cell proliferation, and induction of apoptosis in the renal cortex with exposure concentrations that induced kidney tumors in the cancer bioassay, lends support to the involvement of these mechanisms in THF-induced rat kidney tumors. However, no increase in renal tubule hyperplasia or mineralization was observed in the NTP (1998) study. The detection of α_{2u}-globulin accumulation only when sensitive detection methods were used (i.e., immunohistochemical staining as opposed to standard staining for histopathological examination) suggests that the responses are weak (Chhabra et al., 1998;

NTP, 1998). Furthermore, the observed cell proliferation response, which was increased to a maximum of 298% of controls when selected for focal areas of proliferation, was minimal when compared with cell proliferation responses induced by other well-characterized inducers of α_{2u}-globulin accumulation (Gamer et al., 2002; BASF, 2001a; U.S. EPA, 1991b). There is also an uncertainty regarding the specificity of the relationship between cell proliferation (a putative tumor precursor event) and the observed α_{2u}-globulin accumulation, since the mode of action study by Gamer and colleagues (Gamer et al., 2002; BASF, 2001a) did not include a similar analysis of cell proliferation in female rat kidneys. A major area of uncertainty arises from the absence of detectable histopathological lesions characteristic of this mode of action. No treatment-related renal histopathology or hyaline or granular casts were noted in the BASF study (Gamer, et al., 2002; BASF, 2001a). Because of the weak response in α_{2u}-globulin accumulation and cell proliferation and the absence of the detectable pathological findings, the evidence for this mode of action is equivocal.

Other possible modes of action.

It is possible that advanced CPN may play a role in kidney toxicity and perhaps THF-induced kidney tumors in male rat kidneys. CPN is an age-related renal disease of laboratory rodents that occurs spontaneously and generally with high incidence. Accelerated tubular cell degeneration and regeneration associated with CPN could be involved in the development of proliferative lesions observed in the kidneys of THF-exposed rats. These slight increases in cell proliferation may have contributed to the development of adenomas in male rats in the chronic cancer bioassay.

A comprehensive review that analyzed renal tubule findings from NTP/National Cancer Institute (NCI) bioassays for 69 chemicals, including THF, stated that while the NTP criteria differ in descriptive detail from those of the Society of Toxicologic Pathology, in practice, the actual diagnoses of atypical (focal) tubule hyperplasia (ATH), adenoma, and carcinoma are usually in accord (Lock and Hard, 2004; Hard et al., 1995). Additionally, in a study that examined the utility of multiple-section kidney sampling in the histopathologic evaluation of several NTP bioassays, renal tubule hyperplasia, also termed in the same study as focal renal tubule hyperplasia or focal hyperplasia was differentiated as a potentially preneoplastic lesion that is distinguished from the background regenerative changes of the tubule epithelium that accompany renal toxicity or the common age-related degenerative diseases of the kidney in rats and mice (Eustis et al., 1994). In the same study by Eustis et al. (1994), focal hyperplasia, adenoma, and carcinoma of the renal tubule were considered to constitute a morphological continuum in the development and progression of neoplasia, and that other hyperplastic lesions, specifically focal oncocytic hyperplasia and oncocytoma, were not combined with rat renal tubule hyperplasia because their histogenesis were considered uncertain.

The Society of Toxicologic Pathology Hyperplasia Working Group evaluated the contribution of hyperplastic lesions in 2-year rodent carcinogenicity studies to human hazard identification and risk assessment (Boorman et al., 2003). While acknowledging that ATH is generally considered a preneoplastic lesion, the Society of Toxicologic Pathology asserted that the appearance of neoplasms is the only conclusive evidence of a carcinogenic response and that qualitative evaluation of hyperplastic lesions is more appropriate than statistical analysis. It is not appropriate to combine hyperplastic and neoplastic lesions for statistical analysis (Boorman et al., 2003). The incidences of renal tubule tumors were separated from the findings of ATH although consideration was given, in a qualitative sense, to supporting information from hyperplasia (Lock and Hard, 2004).

In a study aimed at discriminating lesions common to advanced CPN from those that are precursors of renal tubule neoplasia, namely ATH, several archived NTP carcinogenicity studies, including THF, were re-evaluated (Hard and Seely, 2005). Hard and Seely (2005) reported foci of ATH were considered synonymous with renal tubule hyperplasia in NTP reports; simple tubular hyperplasia would not be considered preneoplastic but rather associated with CPN. Utilizing the criteria described in Hard and Seely (2005), a Pathology Working Group (PWG) convened to review the NTP (1998) 2-year inhalation carcinogenicity study of THF (Bruner et al., 2010; PWG, 2009). The PWG pathologists confirmed the NTP conclusions that renal cell adenomas were increased (not statistically significant) in high exposure male rats compared to controls. However, the PWG concluded that when including only ATH as preneoplastic, the incidence of combined preneoplastic and neoplastic lesions was similar between treated and control male rats and further noted that neither α_{2u}-globulin nor CPN-related regenerative processes could be ruled out in THF-induced renal tumors (Bruner et al., 2010).

There was no difference in the incidence or severity of CPN in male rats in the NTP (1998) 2-year carcinogenicity study of THF (both the control and high-exposure groups had the same incidence of end-stage renal CPN). Specifically, against a background of nephropathy that was uniform across all groups, there were more renal tubular tumors in treated rats than in the controls, and those in the higher concentration group animals were larger in size (NTP, 1998). Although THF did not exacerbate development of CPN, it was postulated that it may have exacerbated the development of proliferative lesions within CPN-affected tissue. Taken together, the data are equivocal, and based on the available evidence this potential MOA is not sufficiently supported.

Conclusions about the hypothesized modes of action.

Generally, kidney tumors observed in cancer bioassays are assumed to be relevant for assessment of human carcinogenic potential. However, for male rat kidney tumors, when the mode of action evidence convincingly demonstrates that the response is secondary to α_{2u}-globulin accumulation, the tumor data are not used in the cancer assessment (U.S. EPA, 1991b). There are some data suggesting that male rat kidney tumors, following the inhalation exposure observed in the NTP (1998) bioassay, may be due to the accumulation of α_{2u}-globulin. The criteria for demonstrating this mode of action for risk assessment purposes have been described (U.S. EPA, 1991b). Three core criteria are considered to be most important: (1) increase in hyaline droplets in the renal proximal tubule cells; (2) determination that the accumulating protein in the droplets is α_{2u}-globulin; and (3) presence of additional pathological lesions associated with α_{2u}-globulin. Review of the mode of action data of THF indicates that criteria (1) and (2) are met but criterion (3) is not. An area of uncertainty is the absence of detectable histopathological lesions characteristic of this mode of action (BASF, 2001a; NTP, 1998). The specificity of the response is also difficult to ascertain in the absence of an evaluation of potential α_{2u}-globulin accumulation or other potential precursor events (e.g., cell proliferation) in female rats. However, no increased incidence of kidney tumors was observed in female rats in the NTP (1998) study. Thus, the mode of carcinogenic action of THF-induced renal tumors has not been established.

4.7.3.2. *Liver Tumors*

Description of the hypothesized mode of action.

Induction of a cell proliferation response in the liver by chemicals is generally considered a possible mode of action for liver tumorigenesis that can occur in rodents. Sustained increase in cell proliferation may lead to the promotion of growth of preinitiated cells and subsequently to tumorigenesis. Changes in cellular apoptosis rates can also impact the net rate of tissue growth. Key events for this mode of action may include histopathological evidence of cytotoxicity/necrosis, regenerative growth, and/or apoptosis. Some experimental data suggest that the development of liver tumors in female mice following exposure to THF may involve a cell proliferation-related mode of action; therefore, an analysis of the data is outlined below.

Experimental support for the hypothesized mode of action.

Strength, consistency, specificity of association.

BASF (1998) evaluated the liver tissues from female mice from the NTP (1998) study to examine the relationship between cell proliferation responses and increase in tumors observed in these tissues following THF administration. Histopathological examination and evaluation of cell proliferation as measured by PCNA staining was conducted for tissue samples from the 0, 200, 600, and 1,800 ppm (0, 590, 1,770, and 5,310 mg/m^3) exposure groups (10/group) from the NTP (1998) subchronic (13 weeks) study. No treatment-related histopathology was observed in the female mouse liver tissues. The cell proliferation index was increased (39% over controls) in tissues from the high-concentration exposed mice. However, this result was not statistically significant and was noted as being predominantly based on the results from 2/10 animals. Furthermore, no clear concentration-response pattern was observed, and a significant decrease in proliferation index was observed in the mid-concentration group. Based on these results, the study authors concluded that the examination of the tissues from the 13-week NTP (1998) study revealed no clear increase in cell replication that could be correlated to a tumorigenic mechanism. Gamer and colleagues (Gamer et al., 2002; BASF, 2001a) evaluated a series of endpoints in female B6C3F$_1$ mice (10/group plus 5 in the control and high-concentration enzyme assays) in liver tissues in a short-term, repeated exposure study. Test animals were exposed "nose only" to average THF concentrations of 0, 598, 1,811, or 5,382 mg/m^3 (0, 199, 604, or 1,794 ppm), corresponding to the concentrations used in the NTP (1998) cancer bioassay. Concentrations adjusted for continuous exposure were 0, 107, 323, or 961 mg/m^3. For the animals in each of the four concentration groups, a full necropsy was done, including histopathological evaluation of the liver in addition to measurements of cell proliferation (S-phase response by BrdU staining) and a TUNEL apoptosis assay in the same organ. Since chemical exposures can have varying affects in different regions of the liver lobule, cell proliferation was evaluated separately for zone 1 (periportal, the region adjacent to the portal triad), zone 3 (centrilobular, the region adjacent to the central vein), and zone 2 (midzonal, the area of the lobule intermediate between zones 1 and 3).

THF exposure appeared to induce cell proliferation (see Table C-4) in the female mouse liver. Increased cell proliferation was observed in zones 2 and 3 of the liver of the high-exposure mice following THF exposure for 5 days and in zone 3 following 20 exposures. Coincident with the increase in BrdU labeling, the mitotic index was increased in zone 3 after 5 or 20 exposures in the high-concentration groups. No concentration-dependent increase in BrdU labeling was observed in the animals given a 21-day recovery period, suggesting that the increases in cell proliferation may be an adaptive effect. No treatment-related change in the number of liver cells undergoing apoptosis was observed.

Dose-response concordance.

Gamer and colleagues (Gamer et al., 2002; BASF, 2001a) reported increased cell proliferation following short-term inhalation exposures at concentrations corresponding to those that were tumorigenic in the NTP (1998) bioassay. Therefore, this event appeared consistent with the expected dose response as compared to the tumor outcome.

Temporal relationship.

Gamer and colleagues (Gamer et al., 2002; BASF, 2001a) reported increased cell proliferation in the liver of the high-exposure female mice following short-term inhalation exposures (5 or 20 days) of THF. However, no concentration-dependent increase in BrdU labeling was observed in the animals given a 21-day recovery period.

Biological plausibility and coherence.

Although increased cell proliferation was noted in short-term mode of action studies, the data are not adequate to identify key events that precede this effect. In the earlier of these two mode of action studies (Gamer et al., 2002; BASF, 2001a) it was not clear if the lower degree of BrdU staining after 20 exposures as compared to 5 exposures (see Table C-4) represented fluctuation around an average increase in cell proliferation or a decrease in the rate of proliferation with continued exposure. While the observation that the mitotic index did not similarly decrease after 20 exposures supports the former conclusion, the absence of a significant increase in cell proliferation in tissues obtained from the subchronic NTP (1998) study as reported by BASF (1998) suggests that cell proliferation might not be a sustained response even with continued dosing and fails to explain the late onset of tumors. In the NTP (1998) bioassay, no clear concentration-dependent increase in necrosis was observed, although the incidence of necrosis appeared slightly elevated at the high exposure concentration. Gamer and colleagues (Gamer et al., 2002; BASF, 2001a) reported no histopathological evidence of cell degeneration at concentrations that induced cell proliferation. Other in vitro studies did not suggest that THF is cytotoxic (Matthews et al., 1993; Dierickx, 1989; Curvall et al., 1984). Taken together, these data indicate that THF-induced cell proliferation is not secondary to regenerative hyperplasia.

Changes in cellular apoptosis rates can also impact the net rate of tissue growth. However, the single study that evaluated this endpoint (Gamer et al., 2002; BASF, 2001a) suggested that THF exposure has little impact on apoptosis in the livers of female mice. Therefore, the available data are not sufficient to determine key events associated with cell proliferation that would likely be involved in carcinogenesis.

Other possible modes of action.

One possible mode of carcinogenic action is the ability of THF to inhibit gap junctional intercellular communication (GJIC). In a study by Chen et al. (1984), co-cultures of 6-thioguanine-sensitive and resistant Chinese hamster V79 fibroblast cells were treated with

THF, and the degree of metabolic cooperation was determined by the survival of the resistant cells. The killing of resistant cells serves as an indicator of metabolic cooperation because the toxic 6-thioguanine metabolite that is formed only in the sensitive cells can be passed on to normally resistant cells when gap junctions are intact. Therefore, robust growth of the resistant cells in this assay system would suggest that GJIC is inhibited. THF was judged to be positive (as defined by at least a doubling in recovery of resistant colonies) in the metabolic cooperation assays, suggesting that THF can inhibit GJIC. The recovery rate of resistant cells increased with increasing concentration (up to 100 μL of THF/5 mL of medium). Although there appears to be a correlation between inhibition of GJIC and mouse liver carcinogenesis by some nongenotoxic carcinogens, the mechanism is unclear (Klaunig et al., 1998). The data on GJIC presented by Chen et al. (1984) are too limited to establish that this is the mode of action for the liver tumor induction of THF.

As the major metabolite of THF, GHB, can be converted to GABA, and it has been hypothesized that the production of GABA from THF may perturb the cellular level of putrescine (1,4-diaminobutane), since putrescine is the primary source of GABA in many tissues. Putrescine is required for proper functioning of the cell cycle and for cell growth (Lopez et al., 1999) and has been shown to induce cell transformation and stimulate the expression of *c-fos*, a proto-oncogene (Tabib and Bachrach, 1999). Therefore, it is possible that THF exposure would increase tissue levels of GABA and putrescine, which in turn might promote cell growth and carcinogenesis. However, the link between GABA and putrescine has not been investigated. While this mode of action provides a possible basis for THF-induced cell proliferation and subsequent carcinogenesis, it has not been investigated directly for THF.

Conclusions about the hypothesized mode of action.

Although increased cell proliferation was noted in short-term studies, the data are not adequate to support the hypothesized mode of action. The absence of a significant increase in cell proliferation in tissues obtained from the subchronic NTP (1998) study suggests that cell proliferation might not be a sustained response even with continued dosing. Therefore, while the cell proliferation event meets the requirement of showing the expected temporal relationship at early time points, it is not clear that the effect is sustained for a sufficient duration to adequately explain the late onset of tumors. Furthermore, key precursor events linked to observed cell proliferation have not been identified. The data on other potential modes of action are too limited to establish a mode of action for the THF-induced liver tumors.

4.8. SUSCEPTIBLE POPULATIONS AND LIFE STAGES

4.8.1. Possible Childhood Susceptibility

No adequate studies on the potential reproductive or developmental toxicity of THF in humans were available. However, these endpoints have been evaluated following oral and inhalation exposures to THF in animal studies and oral studies with THF metabolites. A one-generation screening assay (BASF, 1994) and a more comprehensive two-generation assay (Hellwig et al., 2002; BASF, 1996) were conducted for THF administered in the drinking water of rats. Decreased body weights in both male and female pups and delayed eye opening and increased incidence of sloped incisors in F2 pups were observed. There are no data that indicate why developmental delays in eye opening are observed in male pups but not female pups. These developmental effects were observed at doses that also induced maternal effects (although the maternal effects were only of minimal severity). However, as noted in Section 3.1.1.4 of the EPA *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991a), developmental effects occurring at the same exposure levels as maternal toxicity are still considered to represent developmental toxicity and should not be discounted as being secondary to maternal toxicity.

For the THF metabolite GBL, no maternal or developmental effects were observed in rats (Kronevi et al., 1988). Since no effects were observed, this study is not informative in comparing relative susceptibility of adult and young animals. Decreased testicular weight was reported in a short-term reproductive study for GBL (Debeljuk et al., 1983), but no impairment of fertility was reported in the oral two-generation study for THF (Hellwig et al., 2002; BASF, 1996). Developmental studies by the inhalation route have been conducted in both rats (Mast et al., 1992; DuPont Haskell Laboratory, 1980) and mice (Mast et al., 1992). Mast et al. (1992) reported decreased fetal survival and incidence of sternal ossification in mice and decreased fetal body weight in rats. DuPont Haskell Laboratory (1980) reported decreased fetal weight and skeletal alterations. In these inhalation studies, developmental effects were observed at concentrations that also induced maternal toxicity, but not considered secondary to maternal toxicity (U.S. EPA, 1991a).

Comparisons of maternal to developmental effect levels can be useful for evaluating the susceptibility of young animals. The inhalation data for THF suggest that fetuses are not likely to be more susceptible than adult animals. This conclusion is supported by the observation that in the inhalation toxicity database (see Table 4-10) the LOAELs for systemic toxicity in adult animals are significantly lower than the LOAELs for developmental toxicity. In the only available multigeneration study for THF, postnatal development (decreased pup body weight gain, delayed eye opening, and increased incidence of sloped incisors) was affected at drinking water concentrations that had minimal effects on the dams. The results from the two-generation study indicate that the early postnatal period is a period of increased susceptibility, but this conclusion is uncertain since the changes in pup body weight gain may be explained by effects on maternal water intake. Furthermore, the related measure of fetal weight at the end of the prenatal period was not affected in the inhalation developmental studies or in the oral developmental study for GBL.

Only one study was identified that specifically evaluated the effect of age on toxicity of THF. Kimura et al. (1971) estimated oral median lethal dose (LD_{50}) values for a variety of solvents—including THF—for newborn, 14-day-old, young adult, and older adult rats. The oral LD_{50} values for THF were estimated at 2.3 mL/kg for 14-day-old rats, 3.6 mL/kg for young adult rats, and 3.2 mL/kg for older adult rats; none of these values were statistically different. However, the authors report that the newborn animals were much more susceptible than the other age groups, in which doses of 1 mL/kg of all the solvents tested were generally fatal. Since sensitivity was increased in newborns for all the solvents tested, it is not clear whether the increased sensitivity to THF was due to its inherent toxicity to newborn rats or whether some other aspect of the study protocol was responsible. The study results suggest that young animals are at best marginally more susceptible to oral THF exposure than adult animals to high-dose effects.

No toxicokinetic data are available to evaluate potential childhood susceptibility. As a result, the role of age-dependent differences in THF metabolism could not be evaluated. It is important to note, however, that in addition to possible genetic variability (polymorphism) as discussed in Section 4.8.3, age-dependent variability may also exist among key THF-metabolizing enzymes including CYP450 and lactonase (PON1).

The overall data are not sufficient to conclude with certainty whether children are likely to be more susceptible to THF toxicity than adults. Adequate studies directly testing the systemic effects of THF in animals of different ages, as well as data on relevant metabolic parameters are lacking. However, the occurrence of developmental toxicity only at maternally toxic doses suggests that children may not be more susceptible to THF than adults.

4.8.2. Possible Gender Differences

No adequate human studies on gender-based differences in THF toxicity are available. Several toxicity studies of acute, subchronic, or chronic duration in animals have evaluated the toxicity of THF in both males and females administered similar doses. In general, a similar spectrum of noncancer endpoints and effect levels has been observed in both sexes for oral (Hellwig et al., 2002; BASF, 1996; Komsta et al., 1988) and inhalation (NTP, 1998; DuPont Haskell Laboratory, 1996b) exposure studies. However, in the NTP (1998) subchronic study, uterine histopathology changes were observed in mice, but no histopathological effects on the uterus were noted in the companion chronic bioassay (NTP, 1998) or in a short-term inhalation study that evaluated histopathology of the uterus (BASF, 2001a). Changes in uterine weight (not statistically significant) were reported in the short-term study (BASF, 2001a).

In addition, a comprehensive toxicokinetics study of THF following oral dosing of rats and mice of both sexes was conducted by DuPont Haskell Laboratory (1998). The AUC was higher in males, and the corresponding clearance of THF-associated radioactivity from the blood was lower in males of both species. This result might suggest that there are gender differences in THF metabolism, since absorption and distribution of THF were similar for males and females. The available data suggest that THF metabolism is extensive and that oxidative metabolism is due to CYP450 isozymes. However, the identities of the isozymes responsible for THF metabolism have not been elucidated. In vitro evidence suggests that there are species differences in THF metabolism (DuPont Haskell Laboratory, 2000), and, therefore, the differences in THF metabolism between male and female rodents cannot be used to infer the relationship in THF metabolism between sexes in humans. As noted above, whether THF or one of its metabolites is responsible for each of the observed toxic effects has not been demonstrated. As a result of these considerations, the implications of sex-based differences in metabolism cannot be determined.

A significant gender difference in response observed following exposure to THF is the sex-specific induction of kidney tumors in male rats and liver tumors in female mice (see Section 4.7.2), although the absence of an effect in male mice may be due to the apparently higher susceptibility to narcosis (and resulting mortality) in male mice in the chronic inhalation bioassay (NTP, 1998).

The overall similarity in noncancer toxicity between male and female rodents in a variety of bioassays and the absence of functional effects on male or female fertility suggest that gender-based differences in susceptibility to THF are likely to be limited. However, a number of findings raise questions about the potential for increased susceptibility based on gender, including potential effects in the uterus of mice, apparent sex-specific tumor formation, and toxicokinetic differences between male and female rodents.

4.8.3. Other

Possible genetic variability (polymorphism) and/or age-dependent variability in key THF metabolizing enzymes may contribute to interindividual variability in toxicokinetics and possibly to increased sensitivity to THF among certain individuals within the population. As discussed in Section 3.3, the oxidative metabolism of THF to GBL may be catalyzed by one or more of the liver microsomal CYP450 isoenzymes which may be subject to interindividual variation due to genetic polymorphism. Recent investigations have shown that all members of the PON family (e.g., PON1, PON2, and PON3), which are known to be highly conserved in mammals, have lactonase activity and that lactones, including GBL are the preferred substrates for hydrolysis by these isoenzymes (Draganov et al., 2005; Khersonsky and Tawfik, 2005; Billecke et al., 2000). The THF metabolite, GBL, may undergo further metabolism to GHB by the lactonase PON1 enzyme, which also has been known to have genetic variability (polymorphism) in expression and activity of up to 13-fold (van Himbergen et al., 2006); including a possible link to oxidative stress and cardiovascular risk (Bhattacharyya et al., 2008). Additionally, lifestyle factors such as smoking and alcohol consumption may also affect PON1 activity (van Himbergen et al., 2006). It is not clear if and to what extent genetic variability in CYP450 and PON1 may influence the respective oxidative metabolism of THF to GBL or the metabolism of GBL to GHB, and how, in turn, such variability might influence human risk to THF exposure. In addition to possible variability in THF toxicokinetics due to genetic polymorphism of key metabolizing enzymes, other variables could also contribute to the degree of interindividual variability including hepatic blood flow and compensating metabolic pathways (Ginsberg et al., 2009).

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

5.1.1. Choice of Principal Study and Candidate Critical Effects—with Rationale and Justification

A number of human occupational exposure and case report studies following exposure to THF are available (see Section 4.1), and identified effects on both the CNS and liver. However, these studies are unsuitable for the derivation of the RfD because they do not report levels of exposure to THF. In addition, all of these studies report concomitant exposures to other chemicals including solvents that are potentially neurotoxic.

The oral database for characterizing the potential hazards posed by THF in laboratory animals is limited. A one-generation reproductive toxicity (dose range-finding) study (BASF, 1994) and a two-generation reproductive toxicity study (Hellwig et al., 2002; BASF, 1996) in rats (both in drinking water) exist. Both of these studies identified increased kidney weight in adult rats, and decreased pup body weight gain in pups as sensitive effects. Additionally, the two –generation study also observed delayed eye opening and increased incidence of sloped incisors in F2 pups. The two-generation study was selected as the principal study for the RfD. It is a well-conducted, reproductive toxicity study and is considered to be more appropriate for use as the principal study, compared with the one-generation study, because it used a narrower range of exposure concentrations and larger group sizes, and is the more comprehensive of the two studies. The one-generation study was considered supportive.

Regarding kidney weight effects, increased relative kidney weight was observed at similar doses in the parental F0 males and females in both the one- and two-generation studies (less than 10% of the control mean). Treatment-related effects on absolute kidney weight were not as pronounced. For example, the only group for which both relative and absolute kidney weights were significantly increased (p <0.05) was F0 males in the two-generation study, although smaller increases (that were not statistically significant) were noted in other groups. The observation that, at least for one group, both absolute and relative kidney weights were increased indicate that these changes reflect the effects of THF on the kidney itself and are not due solely to body weight changes. This conclusion is supported by the general absence of an effect of THF on body weight gain in adult animals. Kidney weight changes that were observed in the F0 generation were not accompanied by gross kidney pathology, or clinical chemistry findings consistent with an effect on renal function (in the one-generation study) or by histopathological examination (in the two-generation reproductive toxicity study). In addition, exposure to THF had no effect on absolute or relative kidney weight in F1 generation adults.

Thus, the kidney data were not considered further in the selection of the critical effect for the derivation of the RfD.

Decreases in pup body weight gain in F1 and F2 pups and delayed eye opening and increased incidence in sloped incisors in F2 pups observed in rats of the two-generation reproductive toxicity study were considered candidate critical effects for RfD derivation. The decreases in body weight gain were consistently observed in both the F1 and F2 generation pups, and were most pronounced during PND 7–14. The decreases were dose-related, with a positive test for trend ($p < 0.01$), for both male and female pups of both generations. Specifically, body weight gain was decreased by approximately 10-12% at the high dose in the F1 pups and 3-7% and 10% at the mid and high doses, respectively, in the F2 pups. The body weight gain decreases in F2 pups were accompanied by other developmental delays (i.e., delayed eye opening and increased incidence in sloped incisors). These changes occurred in the absence of significant maternal body weight changes or other overt signs of systemic toxicity. EPA concluded that these endpoints indicated delayed or altered growth following exposure to THF; an effect which is one of four major manifestations of developmental toxicity. These endpoints in pups are considered common markers of an adverse toxicological effect on development and are consistent with the principles and practices of the EPA's *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991a).

On account of the small oral database for THF, alternative approaches for deriving the RfD were considered, including the application of a route-to-route extrapolation approach using the inhalation data and or use of the oral data for metabolites of THF. A human PBPK model has been developed by Droz et al. (1999) to estimate the THF concentrations in the blood, breath, and urine following an inhalation exposure for the purpose of determining biological exposure indices that would equate to an occupational exposure level of 200 ppm THF. Human PBPK models with both oral and inhalation portals of entry have not been developed, and no animal PBPK models have been developed. Also, there are no comparative toxicokinetic or toxicodynamic studies following exposure to THF by the oral route in humans and animals. In the absence of PBPK models that include oral and inhalation routes of exposure, and lacking inhalation absorption efficiency data in humans and rats, a route-to-route extrapolation from inhalation to oral exposure for THF would be highly uncertain (U.S. EPA, 2002; 1994b) and was not considered further for development of the RfD.

The use of metabolite data to calculate a reference value may be appropriate when there are no adequate data for the parent compound or when the data indicate that the active form that induces the critical effect is a metabolite derived from the parent compound. In both cases, the kinetics of metabolism would need to be sufficiently understood in order to calculate the administered dose of parent compound from the target tissue dose of the active metabolite. A basic requirement for using the data on metabolites in a quantitative fashion for the dose-response assessment is a demonstration that the critical effects following THF administration can be attributed to the toxicity of metabolites. While THF metabolites also induce CNS toxicity (narcosis), and may be more potent than THF, it is not known if this is true for other target tissue toxicity, such as liver or kidney, as well as effects on postnatal development since evidence is lacking that these effects are due to the action of THF metabolites. Additionally, it is not known whether first pass hepatic metabolism of THF is or is not a detoxifying event in the absence of information on the roles that the intermediate metabolites may play. The available data suggest that the parent compound may be responsible for THF-induced toxicity. Therefore, the oral data for THF, and not a metabolite, are most appropriate to serve as the basis for deriving the RfD.

5.1.2. Methods of Analysis

The candidate critical effects from the two-generation reproductive toxicity study (Hellwig et al., 2002; BASF, 1996) considered for benchmark dose (BMD) modeling were the F1 and F2 pup body weight gains during PND 7–14, as well as F2 pup delayed eye opening on PND 15 and an increased incidence of sloped incisors. However, visual inspection of the data set for delayed eye opening in F2 pups (Table 4-6) suggested that the results were not amenable to modeling. The data were only available as average percentages of affected pups per litter. The dose-response was not clearly monotonic, with the control group showing a larger delay on average than the two lowest dose groups, although a statistically significantly increased delay was indicated in the highest dose group. In addition, the data showed significant heterogeneity in variances which was not amenable to modeling. For sloped incisors, the data were only available as average percentages of affected pups per litter. Thus, data reported for delayed eye opening in F2 pups on PND 15 and sloped incisors in F2 pups were insufficient to support dose-response modeling, and these endpoints are represented by a NOAEL of 3,000 ppm (385 mg/kg-day) and a LOAEL of 9,000 ppm (974 mg/kg-day). The decreases in body weight gain were consistently observed in both the F1 and F2 generation pups, while the delayed eye opening and sloped incisors were only observed in F2 pups. Thus, decreased pup body weight gain was selected as the critical effect, and the related developmental effects are considered supportive of the decreased pup body weight gain. Table 5-1 summarizes the pup body weight gain data.

Table 5-1. F1 and F2 Pup body weight gain changes for RfD derivation from the two-generation reproductive toxicity study in Wistar rats exposed to THF in drinking water[a]

Generation, sex	Parameter	Concentration (ppm)			
		0	1,000	3,000	9,000
F0 Generation/F1 Pups					
F0 Females	TWA THF intake during gestation and lactation (mg/kg-day)	0	134	381	1071
F1 Male pups	Pup body weight gain (g) PND 7−14[b,c]	17.8 ± 1.15	17.5 ± 1.55	17.2 ± 1.43	15.7 ± 1.65[d]
F1 Female pups	Pup body weight gain (g) PND 7−14 [b,c]	17.3 ± 1.47	17.4 ± 1.72	16.9 ± 1.66	15.6 ± 1.56[d]
F1 Generation/F2 Pups					
F1 Females	TWA THF intake during gestation and lactation (mg/kg-day)	0	129	385	974
F2 Male pups	Pup body weight gain (g) PND 7−14[b,c]	17.4 ± 1.56	17.9 ± 1.98	17.0 ± 1.94	15.6 ± 1.67[d]
F2 Female pups	Pup body weight gain (g) PND 7−14 [b,c]	17.2 ± 1.50	17.1 ± 1.62	16.0 ± 2.41	15.4 ± 1.84[d]

[a]See Table 4-6 for additional details.
[b]Positive test for trend (both male and female pups of both F1 and F2 generations); p-value <0.01.
[c]Mean ± SD, where mean is the litter mean and SD reflects variation between litters.
[d]Statistically significantly different ($p \leq 0.05$) from controls.
TWA = time-weighted average.

Sources: Hellwig et al. (2002); BASF (1996).

Details of the dose-response modeling conducted for each endpoint are presented in Tables 5-2 and B-1 (Appendix B). The modeling was conducted following EPA's draft *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000b), and used Benchmark Dose Software (BMDS) version 2.0 (U.S. EPA, 2008). EPA considered benchmark responses (BMRs) of a 5% change and a 1 standard deviation (SD) change in body weight gain compared with the control. A 5% change in body weight gain is considered for pups by analogy to adult body weight, for which a 10% change is generally recognized to support identification of maximum tolerated doses. In the case of a decreasing response, a 1 SD change from the control mean corresponds to 10% of an exposed population having larger decreases in body weight gain than the lowest 1% of the control group, when the data follow a normal distribution (U.S. EPA, 2000b).

In general, model fit was assessed by a χ^2goodness-of-fit test (i.e., models with p <0.1 failed to meet the goodness-of-fit criterion), visual fit, and the Akaike Information Criterion

(AIC) value (i.e., a measure of the deviance of the model fit that allows for comparison across models for a particular endpoint). Model selection details are provided in Appendix B.

Table 5-2. BMD modeling results for pup body weight gain in the Wistar rat two-generation reproductive toxicity study

Dataset	Selected Model	BMD_5 (mg/kg-day)	$BMDL_5$[a] (mg/kg-day)	BMD_{1SD} (mg/kg-day)	$BMDL_{1SD}$[a] (mg/kg-day)
F1 males, PND 7–14	Linear	448	338	1257	928
F1 females, PND 7–14	Linear	478	359	1559	1152
F2 males, PND 7–14	Linear	416	302	1589	1131
F2 females, PND 7–14	Linear	410	296	1661	1174

[a]$BMDL_X$ = 95% lower confidence limit on the maximum likelihood estimate of the dose corresponding to a 5% change or a 1SD change from the control mean. Results are shown rounded to the nearest mg/kg/day.

Sources: Based on data from Hellwig et al. (2002); BASF (1996).

All of the data sets for pup body weight gain during PND 7–14 showed adequate visual and statistical fit by at least one of the models considered. The dose-response pattern was generally similar across the data sets, with linear models providing the best fit in each case. Based on recommendations from the peer reviewers, EPA considered the candidate points of departure (PODs) associated with a BMR of 1SD for pup body weight gain decreases induced by THF. The candidate PODs associated with a BMR of 1SD, shown in Table 5-2, ranged from 928 to 1174 mg/kg-day. The decreased pup body weight gain in F1 males was selected as the basis for the RfD because these data corresponded to the lowest POD (i.e., 928 mg/kg-day).

5.1.3. RfD Derivation—Including Application of Uncertainty Factors (UFs)

The $BMDL_{1SD}$ of 928 mg/kg-day for reduced pup body weight gain in F1 male Wistar rats exposed throughout gestation and lactation was selected as the POD in the derivation of the chronic RfD (Hellwig et al., 2002; BASF, 1996). The uncertainty factors, selected based on EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002; Section 4.4.5), address five areas of uncertainty resulting in a composite UF of 1,000. This composite uncertainty factor was applied to the selected POD to derive an RfD.

A default UF of 10 was applied for inter-individual variability (UF_H) to account for human-to-human variability in susceptibility in the absence of quantitative information to assess the toxicokinetics and toxicodynamics of THF in humans. Although a human PBPK model based on inhalation exposure of volunteers (Droz et al., 1999) is available, information on the human variability in response to exposure to THF is not available.

A default UF of 10 was applied for interspecies extrapolation (UF_A) to account for uncertainty in extrapolating from laboratory animals to humans (i.e., interspecies variability) because information was unavailable to quantitatively assess toxicokinetic or toxicodynamic differences between animals and humans for THF.

An UF of 1 was applied to account for subchronic to chronic extrapolation (UF_S) because developmental toxicity resulting from a narrow period of exposure was used as the critical effect. The developmental period is recognized as a susceptible life stage when exposure during a time window of development is more relevant to the induction of developmental effects than lifetime exposure (U.S. EPA, 1991a).

An UF of 1 was applied for LOAEL-to-NOAEL extrapolation (UF_L) because the current approach is to address this factor as one of the considerations in selecting a BMR for benchmark dose modeling. In this case, a BMR of a 1 SD decrease from the control mean in body weight gain of F1 male rat pups was selected.

An UF of 10 was selected to account for deficiencies in the oral database (UF_D). The oral database for THF contains a two-generation reproductive toxicity study and a range-finding one-generation reproductive study (Hellwig et al., 2002; BASF, 1996, 1994). The one-generation study did not include a histopathological examination of tissues. The two-generation study provided the results of histopathologic examinations limited to evaluations of the liver, kidney, digestive, and reproductive organs in male and female rats. There are no available human occupational or epidemiological studies or standard toxicity studies via the oral route of exposure. Additionally, the database is lacking developmental toxicity studies. Although route-to-route extrapolation was not possible due to inadequate PBPK models (see Section 3.6 and 5.1.1), the inhalation database was considered. Following inhalation exposure, there are developmental toxicity studies (no two-generation reproductive toxicity studies are available) and chronic and subchronic studies available in rats and mice (NTP, 1998; Mast et al., 1992; DuPont Haskell Laboratory, 1980). The inhalation developmental studies provided evidence of effects on the fetus. The subchronic and chronic studies reported systemic toxicity (CNS effects and liver weight changes) at exposure concentrations lower than those inducing developmental toxicity; suggesting that prenatally exposed fetuses may not be more sensitive than adult animals. Thus, the lack of studies examining endpoints other than reproductive toxicity (i.e., standard toxicity studies examining a comprehensive array of endpoints and developmental studies) following oral exposure is a database deficiency; a 10-fold UF was applied.

The RfD based on the $BMDL_{1SD}$ for decreased pup body weight gain (Hellwig et al., 2002; BASF, 1996) was derived as follows:

$$
\begin{aligned}
RfD &= BMDL_{1SD} \div (UF_H \times UF_A \times UF_D) \\
&= 928 \text{ mg/kg-day} \div (10 \times 10 \times 10) \\
&= 928 \text{ mg/kg-day} \div 1{,}000 \\
&= 0.928 \text{ mg/kg-day} \\
&= \mathbf{9 \times 10^{-1}} \textbf{ (rounded to 1 significant figure).}
\end{aligned}
$$

5.1.4. Previous RfD Assessment

This is the first IRIS assessment for THF; thus, an oral RfD was not previously available on IRIS.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

5.2.1. Choice of Principal Study and Critical Effect—with Rationale and Justification

Human occupational exposure studies and case reports have investigated the health effects following exposure to THF. These studies indicate that the nervous system and liver may be targets of toxicity of THF. However, all of the published human studies contain insufficient data on the duration and/or concentration of THF exposure. In addition, the human exposure studies indicate the potential for coexposure to other solvents. Thus, the available human studies are not considered suitable for use in the derivation of an RfC.

Animal studies are available that examine inhalation effects of THF following subchronic exposure in rats and mice (NTP, 1998; DuPont Haskell Laboratory, 1996b; Malley et al., 2001; Horiguchi et al., 1984; Kawata and Ito, 1984; Stasenkova and Kochetkova, 1963) and 2-year exposure in rats and mice (NTP, 1998), in addition to developmental toxicity studies in both mice (Mast et al., 1992) and rats (Mast et al., 1992; DuPont Haskell Laboratory, 1980). Several of these studies reported portal-of-entry findings, including irritation of the nasal and respiratory tracts (Horiguchi et al., 1984; Kawata and Ito, 1984; Stasenkova and Kochetkova, 1963) but were not considered suitable for RfC derivation due to concerns about lack of consistency among study findings, reporting of these effects, and study design (see Section 4.6.2).

Following chronic exposure, no clinical findings were observed in female mice; however, clinical signs of CNS toxicity (narcosis) were the only effects observed in male mice during and up to 1 hour after cessation of exposure to THF at 5,310 mg/m^3 (NTP, 1998; chronic). Similar effects were observed following subchronic exposure to THF in which CNS toxicity (narcosis) was reported in both male and female rats at 14,750 mg/m^3 THF and mice at $\geq$ 5,310 mg/m^3, respectively (NTP, 1998; subchronic). Immediately after exposure, both male and female rats in the high exposure group showed ataxia (irregular movement with lack of coordination). Male and female mice exposed to 5,310, and 14,750 mg/m^3 were in a state of narcosis (stupor) during exposure, but were alert and fully awake immediately after exposure to 5,310 mg/m^3 while mice in the 14,750 mg/m^3 group required up to 2 hours for recovery (NTP, 1998; chronic). It should be noted that it is possible that the rats and mice may have developed a tolerance to THF exposure considering the effects were observed at similar concentrations (5,310 mg/m^3) in the subchronic and chronic studies by NTP (1998). However, this cannot be determined due to the lack of reporting of incidence data for these effects and because the chronic study did not include the higher exposure group (14,750 mg/m^3) for comparison.

Further support for THF-induced CNS effects is provided by neurotoxicity, developmental, acute, and short-term studies. The only findings in a neurotoxicity study were sedative effects in male and female rats at 4,425 and 8,850 mg/m^3 (DuPont Haskell Laboratory, 1996b; Malley et al., 2001). Developmental studies conducted in both rats and mice reported maternal toxicity including CNS effects (Mast et al., 1992). Following acute and short-term exposure, symptoms of CNS toxicity, including sedation, coma, altered respiration, and decreased response to external stimuli, were observed in dogs (Stoughton and Robbins, 1936), mice (Stasenkova and Kochetkova, 1963; Stoughton and Robbins, 1936), and rats (Horiguchi et al., 1984; DuPont Haskell Laboratory, 1979; Stasenkova and Kochetkova, 1963) (See Appendix C for study descriptions). Additionally, as reported in Section 4.1, human CNS effects may result from THF occupational exposure. Based on the above findings, the CNS toxicity was further considered as a candidate critical effect in the derivation of the RfC.

Chronic exposure to THF resulted in a slight increase in liver necrosis in the 5,310 mg/m^3 exposure group for female mice (from 3/50 in controls to 7/48) (NTP, 1998; chronic). No other non-neoplastic findings were seen in male or female mice or rats following chronic exposure. Subchronic exposure to THF (NTP, 1998; subchronic) provided evidence of increased liver weights (both absolute and relative) in the 14,750 mg/m^3 female rats and this finding was accompanied by increased serum bile acid concentration in the absence of cholestasis or hepatocellular necrosis. The study authors indicated that these changes were consistent with decreased or altered hepatic function. In male mice, absolute and relative liver weights were concentration-dependently and statistically significantly increased (7 – 36% above control) following exposure to concentrations of $\geq$1,770 mg/m^3. The increases in absolute and relative liver weights in male mice were supported by an increased incidence of centrilobular cytomegaly (graded mild), statistically significant at 14,750 mg/m^3 (7/10 compared to 0/10 in the next lower exposure group and the controls). Also, relative and absolute liver weights were significantly increased (6 – 14% above control) in female mice beginning at 5,310 mg/m^3 and were accompanied by centrilobular cytomegaly (graded minimal) at 14,750 mg/m^3 (10/10 animals compared to 0/10 in the next lower exposure group and the controls) (NTP, 1998; subchronic). The affected hepatocytes were additionally described as having slight karyomegaly (enlarged nucleus), increased cytoplasmic volume, and granular cytoplasm with less vacuolation than that of midzonal and periportal hepatocytes (NTP, 1998; subchronic). Liver tissues from male and female mice of the three lower exposure groups were not examined. Additionally, no clinical chemistry measurements were performed in mice; however, the finding of increased bile acids in rats, in the absence of increased serum liver enzymes, was interpreted as possibly signifying decreased or altered hepatocellular function in the 14,750 mg/m^3 exposure group.

Further support in the database exists for liver effects following THF exposure. Specifically, fatty liver degeneration (or infiltration) which was observed following short-term inhalation exposure in female mice (Gamer et al., 2002; BASF, 2001a), is a likely adverse effect since certain drugs which evoke fatty liver changes may predispose the liver to oxidative stress, lipid peroxidation, and possible mitochondrial and organ damage (Begriche et al., 2006; Letteron et al., 1996). In another subchronic inhalation toxicity study, Horiguchi et al. (1984) reported mild liver toxicity in male rats in the form of increased serum liver enzymes, bilirubin, and cholesterol at THF exposure concentrations of 2,950 and 14,750 mg/m^3 in addition to increased relative liver weight at 14,750 mg/m^3 but no liver histopathology findings were reported (Section 4.2.1.2). Some earlier studies also reported liver effects when THF was administered in animals using exposure routes other than inhalation (Komsta et al., 1988; Stasenkova and Kochetkova, 1963). As reported in Section 4.1, the human liver also may be a target organ for THF occupational exposure settings. While the reported liver findings may be confounded by the likelihood of coexposure to other chemicals, it is reasonable to conclude that repeated occupational exposure to high concentrations of THF may have contributed to the large increases in serum liver enzymes and the palpable liver findings in some of the human studies (Garnier et al., 1989; Horiuchi et al., 1967).

Subchronic exposure also resulted in effects including altered organ weights (thymus and spleen), increased bile acids, and altered hematological parameters at 14,750 mg/m^3 THF in male and female rats; however, no histopathological lesions were identified (NTP, 1998; subchronic). The biological significance of the decrease in thymus weight was considered questionable (Section 4.6.2). Degeneration of the adrenal cortex and uterine atrophy in the 14,750 mg/m^3 female mice was also observed. According to the study authors, degeneration of the adrenal cortex and uterine atrophy may have been a direct effect of THF on these tissues or may be the result of a hormonal effect, possibly through perturbation of the pituitary-hypothalamic-end organ axis (NTP, 1998; subchronic). On the other hand, no histopathological effects on the uterus or adrenals were noted in the companion chronic bioassay (NTP, 1998; chronic) or in a short-term inhalation study that evaluated histopathology of the uterus (BASF, 2001a). The effects on the thymus, spleen, adrenal cortex, and uterus were not considered further in the derivation of the RfC due to the lack of response and uncertainty regarding toxicological significance.

In consideration of the available studies reporting effects of chronic and subchronic THF exposure in animals, the NTP (1998) study was chosen as the principal study. The subchronic phase, rather than the chronic phase, of this study was selected to serve as the principal study due to comprehensive reporting in the subchronic study which better characterized the low-exposure effects associated with THF (see Table 5-3 for study design details).

Table 5-3. Subchronic and chronic inhalation toxicity studies of THF in mice and rats (NTP, 1998): Exposure concentrations and examined non-neoplastic parameters

Study	Exposure Concentration	Parameters Evaluated
Subchronic (13-week) study: 10/sex/exposure group of rats or mice	0, 195, 590, 1,770, 5,310, 14,750 mg/m^3	Body weight, clinical observation, clinical chemistry, organ weights, complete histopathology for control and high concentration groups; gross lesions and potential target tissue histopathology for other exposure groups
Chronic (2-year) study: 50/sex/exposure group of rats or mice	0, 590, 1,770, 5,310 mg/m^3	Body weight, clinical observations, tissue histopathology

Source: NTP (1998).

Sensitive endpoints identified in this study, the effects in the CNS and liver, were selected as the co-critical effects. The CNS effects were observed in rats and mice (at concentrations ≥5,310 mg/m^3) and the liver effects were observed in rats (at concentrations of 14,750 mg/m^3) and mice (at concentrations ≥590 mg/m^3). The toxicological significance of the observed liver weight changes was considered to be uncertain at the low concentrations (590-1,770 mg/m^3), where the changes were of minimal severity and were not accompanied by other signs of liver toxicity. The increases in absolute and relative liver weights at 5,310 mg/m^3 were greater than 10% above controls (statistically significant) and were accompanied by minimal increases in histopathology findings (1/10 incidence in centrilobular cytomegaly) that progressed with increases in THF concentration. The liver and CNS effects observed at the exposure concentration of ≥5,310 mg/m^3 were considered biologically significant and representative of adverse effects (U.S. EPA, 2002; 1998).

5.2.2. Methods of Analysis

The most relevant endpoints for deriving the POD for the quantitative assessment were CNS effects, hepatic centrilobular cytomegaly, and increased liver weights in male mice in the NTP (1998) subchronic study. Data in mice, rather than rats, were modeled because mice were more sensitive to the THF-induced liver and CNS effects. The selection of the male mouse data was based on the fact that the liver weight increased more steadily from lower administered exposure in males than in females. Suitable data were available to model the liver weight and liver histopathology findings using dose-response modeling methods (see Table 5-4). Note that because there was very little effect on body weight until the highest exposure, the absolute and relative liver weight changes were essentially the same, and only the absolute liver weights were considered for modeling. For CNS effects, no incidence data were available from the NTP (1998) study, therefore dose-response modeling could not be conducted for this endpoint, and a NOAEL was identified for the candidate POD. See Table 5-4 for the data considered for candidate POD derivation for the liver effects.

Human equivalent concentrations (HECs) for the potential critical effects were derived (Section 5.2.2.1), and the final selection of the POD for the RfC was made after the evaluation of effect levels among multiple endpoints from the principal study (Section 5.2.2.2).

5.2.2.1. *Calculation of HECs*

The *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (hereafter referred to as the RfC Methodology) recommends converting the $POD_{[ADJ]}$ (ADJ, adjusted for continuous exposure) to a human equivalent concentration (HEC) (U.S. EPA, 1994b). For the purposes of this assessment, the induction of extrarespiratory tract effects in the liver and CNS is consistent with properties of a category 3 gas as described under the RfC methodology (U.S. EPA, 1994b).

For category 3 gases, HECs are calculated by multiplying the duration-adjusted exposure concentration by the regional gas dose ratio (RGDR) for the extrarespiratory region. The RGDR for extrarespiratory effects is calculated by finding the ratio of the animal-to-human blood:gas (air) partition coefficients. In cases where there are either no data available or where the animal partition coefficient is larger than the human coefficient, a default value of 1 is used for the RGDR. For THF, a human blood:gas partition coefficient was available from Ong et al. (1991); however, no value was available for animals. Therefore, the default of 1 was applied in estimating the HECs for extrarespiratory effects. For example, for the concentration of 1,770 mg/m^3, which corresponds to the NOAEL for the CNS toxicity (narcosis) in male and female mice in the NTP (1998) study, the HEC based on the equation for a category 3 gas was calculated by estimating continuous equivalent exposure and applying the RGDR, as follows:

$$NOAEL_{adj} = 1{,}770 \text{ mg/m}^3 \times 6/24 \text{ hours} \times 5/7 \text{ days} = 316 \text{ mg/m}^3$$

$$NOAEL_{HEC} = 316 \text{ mg/m}^3 \times \text{default RGDR of } 1 = 316 \text{ mg/m}^3$$

The HECs calculated for each study concentration were used directly in conducting the benchmark concentration (BMC) modeling of the liver effects. See Table 5-4 for the estimated HECs.

Table 5-4. Measures of liver toxicity in B6C3F$_1$ male mice following subchronic inhalation exposure to THF

	Administered Concentration in ppm (concentration in mg/m^3)					
	0 (0)	**66 (195)**	**200 (590)**	**600 (1770)**	**1,800 (5,310)**	**5,000 (14,750)**
	Human Equivalent Continuous Concentration (mg/m^3)					
Endpoint	**0**	**35**	**105**	**316**	**948**	**2,634**
Absolute liver weight (g) [a]	1.613 ± 0.037	1.667 ± 0.022	1.695 ± 0.037	1.722 ± 0.031[b]	1.789 ± 0.035[c]	1.964 ± 0.060[c]
Centrilobular cytomegaly	0/10	NE	NE	NE	1/10	7/10

[a]Mean ± standard error. All group sizes are 10 animals/group except for male mice in the 5,000 ppm group where N = 7.
[b]$p \leq 0.05$.
[c]$p \leq 0.01$.
NE = Not examined.

Source: Adapted from NTP (1998).

5.2.2.2. *Dose-Response Modeling*

The dose-response modeling was conducted following EPA draft BMD technical guidance (U.S. EPA, 2000b) and using BMDS version 2.0 (U.S. EPA, 2008) similar to methods utilized for the RfD (see Section 5.1.2). For liver weights, a BMR of a 10% change relative to control mean was used, by analogy to its use in evaluating adult body weight changes. In addition, a BMR of 1 SD was also estimated for each endpoint for comparison purposes (see Appendix B). For centrilobular cytomegaly, no biological criterion for defining adversity was available, and a 10% extra risk was used under the assumption that is represents a minimal, biologically significant effect level.

For the liver weight data set, all of the continuous models fit the data adequately (see Table B-3, Appendix B). BMCLs ranged over fourfold, leading to the selection of the unrestricted power model, with the lowest BMCL, for providing the candidate POD (see

Appendix B). The EPA's BMD technical guidance has generally recommended restricting the power parameter in the power model to be greater than 1, primarily to avoid low-dose extrapolation in regions where the estimated dose-response relationship is so steep that it may appear biologically implausible. For these data, however, the BMRs of 10% change relative to the control mean and 1 SD both fell well within the data range, and BMCLs estimated with unrestricted parameters provide more accurate confidence interval coverage. The candidate POD for increased absolute liver weight was the BMCL (95% lower bound on the BMC) of 246 mg/m^3 (Table 5-5).

For the centrilobular cytomegaly data set, the full suite of quantal models in BMDS was considered. All of the models provided an adequate fit overall to the data set based on a goodness-of-fit *p*-value >0.1. Of the models exhibiting adequate fit, BMCLs fell within a threefold range, and the model yielding the lowest AIC value for a data set was selected as the best-fit model (U.S. EPA, 2000b). The multistage model demonstrated the lowest AIC (Table B-3). The candidate POD for centrilobular cytomegaly was the BMCL of 256 mg/m^3 (Table 5-5).

Table 5-5. BMC[a] modeling results for noncancer liver effects in male mice resulting from subchronic inhalation exposure to THF

Dataset	Selected Model	BMC_{10}[b]	$BMCL_{10}$[b]
Absolute liver weight	Power (unrestricted)	783	246
		BMC_{10}	$BMCL_{10}$
Centrilobular cytomegaly	Multistage, degree 2 (coefficients ≥0)	805	256

[a]Concentrations used in the modeling were the HECs in mg/m^3 (see Table 5-4).
[b]For liver weights, BMC_{10} and $BMCL_{10}$ refer to a BMR of 10% increase over the control mean, while for centrilobular cytomegaly, BMC_{10} and $BMCL_{10}$ refer to 10% extra risk.

Data Source: Based on data from NTP (1998).

For CNS effects in male and female mice, no incidence data were available, and a NOAEL of 1,770 mg/m^3 was identified as the POD. The adjustment for human equivalent continuous concentration corresponds to a candidate POD of 316 mg/m^3. The liver effects (increased liver weight and cytomegaly) and CNS effects in male mice are considered co-critical effects and the PODs for these effects are similar, ranging from 246-316 mg/m^3. The lowest of these three PODs, the $BMCL_{10}$ of 246 mg/m^3 based on findings of increased absolute liver weight in male mice, was used to calculate the RfC because it was the most sensitive endpoint. However, a derivation of a potential RfC based on the POD for CNS toxicity, the $NOAEL_{HEC}$ of 316 mg/m^3, is presented in Section 5.2.3 for comparison purposes.

5.2.3. RfC Derivation—Including Application of Uncertainty Factors (UFs)

The $BMCL_{10}$ of 246 mg/m^3 for increased absolute liver weight in male $B6C3F_1$ mice exposed to THF for 6 hours/day, 5 days/week for 90 days (NTP, 1998) was selected as the POD in the derivation of the RfC. The uncertainty factors, selected based on EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002; Section 4.4.5), address five areas of uncertainty resulting in a composite UF of 100. This composite uncertainty factor was applied to the selected POD to derive an RfC.

A default UF of 10 was applied for inter-individual variability (UF_H) to account for human-to-human variability in susceptibility in the absence of quantitative information to assess the toxicokinetics and toxicodynamics of THF in humans. Although a human PBPK model based on inhalation exposure of volunteers (Droz et al., 1999) is available, information on human variability relating to toxicodynamics and toxicokinetics in response to exposure to THF is not available.

An UF of 3 (i.e., $10^{1/2}$ = 3.16 rounded to 3) was applied for interspecies extrapolation (UF_A) to account for the uncertainty in extrapolating from laboratory animals to humans. This value is adopted by convention where an adjustment from an animal-specific POD_{ADJ} to a POD_{HEC} has been incorporated. Application of an UF of 10 would depend on two areas of uncertainty (i.e., toxicokinetic and toxicodynamic uncertainties). In this assessment, the toxicokinetic component associated with exposure to THF is mostly addressed by the determination of an HEC as described in the RfC methodology (U.S. EPA, 1994b). The toxicodynamic uncertainty is also accounted for to a certain degree by the use of the applied dosimetry method and an UF of 3 is retained to account for the remaining uncertainty regarding the toxicodynamic differences between mice and humans.

An UF of 1 was applied to account for extrapolation from subchronic-to-chronic exposure (UF_S), due to the lack of evidence that increased duration of exposure to THF may increase the incidence or severity of these effects. The 14-week study for THF (NTP, 1998; subchronic), selected as the principal study, reported critical findings of CNS effects and increased liver weight which was supported by hepatic centrilobular cytomegaly. In the chronic exposure phase of the study, while no organ weights were taken, no hepatic cytomegaly was identified at any exposure level including the high exposure group of 5,310 mg/m^3. However, the incidence of liver necrosis in the female mice of the 5,310 mg/m^3 exposure group (NTP, 1998; chronic) was increased (although not statistically significant) from 3/50 in the control to 7/48. The available chronic information suggests that liver damage observed in rodents following subchronic exposure to THF (NTP, 1998) may not progress to more severe effects following chronic exposures near the POD, considering that cytomegaly was not reported at chronic exposures $\leq$ 5,310 mg/m^3 and that necrosis was only observed at 5,310 mg/m^3 (the highest concentration in chronic study), the same concentration as the LOAEL for the CNS and liver effects in the subchronic study. Additionally, the CNS effects were observed following exposure to 5,310 mg/m^3 in both the subchronic and chronic studies but with no evidence of effects at lower concentrations in the chronic study. A full comparison of the studies is not possible given the incidence data were not reported for these effects in either study. However, the available evidence suggests that increased duration of exposure to THF may not increase the incidence or severity of these effects; thus, an UF of 1 was applied.

An UF of 1 was applied for LOAEL-to-NOAEL extrapolation (UF_L) because the current approach is to address this factor as one of the considerations in selecting a BMR for benchmark dose modeling. In this case, a BMR of 10% change in absolute liver weight in male mice was selected as a minimal biologically significant change.

An UF of 3 was applied to account for deficiencies in the database (UF_D) for THF. Chronic and subchronic inhalation bioassays and developmental toxicity studies are available in rats and mice (NTP, 1998; Mast et al., 1992; DuPont Haskell Laboratory, 1980). The inhalation data for THF (see Section 4.2) suggest that fetuses may not be more sensitive than adult animals given that the observed LOAELs for developmental effects were greater than the LOAELs for systemic toxicity (CNS and liver weight changes) in adult animals (see Table 4-10). In the oral two-generation reproductive toxicity study for THF, postnatal development (decreased pup body weight gain, in addition to delayed eye opening and increased incidence of sloped incisors) was affected at drinking water concentrations that had minimal effects on the dams. No two-generation reproductive toxicity study by the inhalation route is available. Therefore, a database UF of 3 was applied to account for the lack of a two-generation reproductive study.

The RfC based on the $BMCL_{10}$ for increased absolute liver weight (supported by the co-critical effects, comprising CNS effects and increased incidence of centrilobular cytomegaly) in male B6C3F$_1$ mice (NTP, 1998), was derived as follows:

$$
\begin{aligned}
\text{RfC} \quad &= BMCL_{10} \div (UF_H \times UF_A \times UF_D) \\
&= 246 \text{ mg/m}^3 \div (10 \times 3 \times 3) \\
&= 246 \text{ mg/m}^3 \div 100 \\
&= 2.46 \text{ mg/m}^3 \\
&= \mathbf{2\ mg/m^3\ (rounded\ to\ 1\ significant\ figure).}
\end{aligned}
$$

For comparison, a potential RfC can be derived from the POD_{HEC} based on the NOAEL for CNS effects as follows:

$$
\begin{aligned}
\text{RfC} \quad &= \text{NOAEL}_{HEC} \div (\text{UF}_H \times \text{UF}_A \times \text{UF}_D)\\
&= 316\ \text{mg/m}^3 \div 100\\
&= 3.16\ \text{mg/m}^3\\
&= \mathbf{3\ mg/m^3\ (rounded\ to\ 1\ significant\ figure).}
\end{aligned}
$$

5.2.4. Previous RfC Assessment

This is the first IRIS assessment for THF; thus, an inhalation RfC was not previously available on IRIS.

5.3. CANCER ASSESSMENT

Under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), the database for THF provides "suggestive evidence of carcinogenic potential" by all routes of exposure based on an increased incidence of hepatocellular adenomas and carcinomas in female $B6C3F_1$ mice and renal tubule adenomas and carcinomas in male F344/N rats following inhalation exposure to THF. There was no evidence of carcinogenic activity in male mice or female rats reported by NTP (1998), and no other treatment-related increases in tumor incidence were observed. No studies evaluating the carcinogenicity of THF by the oral or inhalation routes were identified in humans (see Section 4.1).

5.3.1. Evaluation of Inhalation Cancer Data

A 2-year NTP (1998) inhalation cancer bioassay reported an increased incidence of hepatocellular adenomas and carcinomas in female $B6C3F_1$ mice (statistically significant trend) and an increased incidence of renal tubule adenomas and carcinomas in male F344/N rats (statistically significant trend) following inhalation exposure to 200, 600, and 1,800 ppm THF (NTP, 1998) (see Section 4.7.2).

The U.S. EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) state: "When there is suggestive evidence, the Agency generally would not attempt a dose-response assessment, as the nature of the data generally would not support one; however, when the evidence includes a well-conducted study, quantitative analyses may be useful for some purposes, for example, providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities. In each case, the rationale for the quantitative analysis is explained, considering the uncertainty in the data and the suggestive nature of the weight of evidence. These analyses generally would not be considered Agency consensus estimates."

In this case, the NTP (1998) cancer bioassay for THF is a well-conducted study showing hepatocellular adenoma or carcinomas in female mice that were increased in a dose-related manner, starting at an approximate 15% increase over controls at the lowest exposure. The adjusted rate of renal adenoma or carcinoma in male rats was also increased in a dose-related manner, starting at an approximate 9% increase over controls at the lowest exposure. The data from this study are amenable to modeling; EPA would generally derive a cancer risk estimate from such data.

However, a majority of the external peer review panel members (see Appendix A: Summary of External Peer Review and Public Comments and Disposition) stated that derivation of an inhalation unit risk (IUR) for THF would result in an overestimation of cancer risk if a linear low-dose extrapolation approach was utilized. Although the reviewers agreed with EPA's conclusion that based on the available data the modes of action for both liver and kidney tumors induced by THF are not well understood, they suggested that THF is a weak, nongenotoxic carcinogen that would have a threshold. Specifically, they stated that THF does not appear to be genotoxic, does not produce irreversible damage and/or proliferative lesions that are preneoplastic, is rapidly metabolized, and is not bioaccumulative, and thus recommended the use of a nonlinear extrapolation approach to quantify cancer risk for THF. The reviewers who recommended a nonlinear approach suggested that a nongenotoxic carcinogen would consequently have a nonlinear cancer response at low dose. This concept is recognized as controversial in the scientific community.

The U.S. EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) recommend that the method used to characterize and quantify cancer risk for a chemical is determined by what is known about the mode of action of the carcinogen and the shape of the cancer dose-response curve. The linear approach is recommended if the mode of action of carcinogenicity is not understood (U.S. EPA, 2005a). In the case of THF, very little data are available to inform the mode of action and no data are available to indicate the shape of the dose-response curve at low doses. If data were available to better inform the mode of action, and the data were indicative of a threshold response, then a reference value could be derived based on a precursor endpoint (i.e., key event in the mode of action) and considered for the RfC for THF. In such a case, the reference value would be considered protective against tumor development following inhalation exposures. For THF, there were no reported noncancer effects that could serve as a precursor endpoint upon which to base a nonlinear analysis. EPA considered whether the cell proliferation reported in the livers of mice following short-term exposure to THF was a potential key event in the development of female mouse liver tumors; however, given the absence of proliferation data in any of the subchronic or chronic studies, the use of this endpoint is not supported. Thus, the nonlinear analysis recommended by the peer reviewers cannot be readily implemented.

Based on the peer reviewers' concern for the potential overestimation of risk in deriving an IUR for THF using a linear low-dose extrapolation approach combined with the uncertainty associated with the carcinogenic potential for THF, EPA did not derive an IUR. However, because there may be some circumstances for which a cancer risk estimate for THF would be useful, EPA has presented what the inhalation cancer risk estimate would be if it were derived using a linear low-dose approach. This derivation can be found in Appendix B. Risk assessors should use caution when considering the use of this value due to the uncertainty associated with the potential overestimation of risk related to the linear low-dose extrapolation approach employed in its derivation and the suggestive nature of the tumorigenic response.

5.3.2. Previous Cancer Assessment

This is the first IRIS assessment for THF; thus, a cancer assessment was not previously available on IRIS.

6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1. HUMAN HAZARD POTENTIAL

6.1.1. Oral Noncancer

The database for oral toxicity of THF is limited. No human data following oral exposure to THF are available. A number of human occupational exposure and case report studies suggesting CNS and liver effects following THF exposure are available; however, these studies do not report levels of THF exposure and all studies included exposures to other chemicals known to cause similar toxicity. CNS toxicity appears to be the primary health effect following acute exposure in animals, although no CNS effects were reported in the rodent drinking water reproductive toxicity studies of longer exposure duration. Short-term and subchronic oral exposure studies (4 weeks to approximately 90 days) suggest that general toxicity (characterized by altered food and water consumption and decreased body weight) and liver and kidney toxicity are potential health effects of oral exposure to THF. The available reproductive toxicity studies suggest that THF induces effects in the offspring of exposed dams.

The two-generation reproductive toxicity in rats (Hellwig et al., 2002; BASF, 1996) was selected as the principal study for the derivation of the RfD. The **RfD of 0.9 mg/kg-day** is based on a $BMDL_{1SD}$ of 928 mg/kg-day for decreased pup body weight gain (Hellwig et al., 2002; BASF, 1996). A composite UF of 1,000 was used. This factor is based on selection of an uncertainty factor of 10 to account for intrahuman variability, 10 for interspecies extrapolation, and 10 for uncertainties in the database.

No sensitive subpopulations have been identified. The developmental effects were observed at doses that also induced maternal effects (although the maternal effects were only of minimal severity). Therefore, the existing data do not provide convincing evidence for age-related differences in sensitivity to noncancer effects following oral exposure to THF. Overall, there is some uncertainty about systemic toxicity following exposure to THF by the oral route. Specifically, the available data on parental rats was limited to body weight, food and water intake, reproductive performance, and histopathology of limited organs. There is uncertainty regarding other non-examined parameters following exposure by the oral route including possible developmental neurobehavioral effects, hematotoxicity, functional immunity, and a more inclusive histopathology assessment.

A confidence level of high, medium, or low is assigned to the study used to derive the RfD, the overall database, and the RfD itself, as described in Section 4.3.9.2 of EPA's *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994). There is medium confidence in the principal study (Hellwig et al., 2002;

BASF, 1996); however, the overall confidence in the oral THF database is low, with several key data gaps identified, including lack of a full systemic toxicity study and developmental toxicity studies. Therefore, the **confidence in the RfD** is characterized as **low-to-medium**.

6.1.2. Inhalation Noncancer

Although no epidemiological studies of THF have been conducted, several occupational exposure case studies in humans suggest that target organs in humans are the CNS, respiratory tract, liver, and kidney (Garnier et al., 1989; Albrecht et al., 1987; Juntunen et al., 1984; Edling, 1982; Emmett, 1976). The major uncertainties associated with all of the reported human case studies are that none reported actual exposure levels for THF and the workers were exposed to other solvents and chemicals in addition to THF; therefore, it is not possible to attribute the observed effects to THF exposure alone. Nevertheless, inhalation studies in animals generally identified a similar array of target organs (see Table 4-10) including clinical signs of CNS toxicity and liver toxicity.

Respiratory tract irritation was reported in multiple human and animal studies. One consideration in evaluating the potential health consequences due to THF-induced respiratory tract irritation is the role of the exposure duration on the severity of the effect. Several acute or short-term exposure studies (Ikeoka et al., 1988; Horiguchi et al., 1984; Ohashi et al., 1983) identified concentrations inducing irritant responses that were lower than the concentrations that induced toxicity in subchronic and chronic studies (NTP, 1998). There is direct evidence that respiratory tract responses are transient in nature, waning with increasing exposure duration (Horiguchi et al., 1984). These data suggest that irritant responses not observed with subchronic or chronic exposure could occur in individuals who were not previously exposed.

Several systemic effects have been observed following subchronic or chronic inhalation exposure to THF. Decreased body weight has been observed in rats (Horiguchi et al., 1984; Kawata and Ito, 1984). Decreased blood pressure was observed in dogs (BASF, 1938) and rats (Stasenkova and Kochetkova, 1963). Altered hematological parameters were observed in rats (NTP, 1998; Horiguchi et al., 1984), mice (NTP, 1998; Stasenkova and Kochetkova, 1963), and dogs (BASF, 1938). Following 14 weeks of inhalation exposure, rats of both sexes had significantly increased relative liver weight and significantly relative weights for thymus and spleen; male rats also had significantly increased relative kidney and lung weights (NTP, 1998). In the same study, mice of both sexes showed increased relative liver weight and decreased relative spleen weight, while male mice only had decreased relative thymus weight and female mice had a slightly reduced relative lung weight (NTP, 1998). In addition, Horiguchi et al. (1984) observed increased relative weights of brain, lung, liver, pancreas, spleen, and kidney.

Developmental studies by the inhalation route have been conducted in both rats (Mast et al., 1992; DuPont Haskell Laboratory, 1980) and mice (Mast et al., 1992). In both studies and both species, maternal toxicity included symptoms of CNS effects and significant decreases in body weight accompanied by decreases in gravid uterine weight (Mast et al., 1992) or food consumption (DuPont Haskell Laboratory, 1980). Decreased fetal weight was observed at the same concentration that resulted in maternal toxicity in rats (Mast et al., 1992). In both mice (Mast et al., 1992) and rats (DuPont Haskell Laboratory, 1980), decreased fetal survival also occurred at the same concentrations that resulted in maternal toxicity. However, as noted in Section 3.1.1.4 of the EPA *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991a), developmental effects occurring at the same exposure levels as maternal toxicity are still considered to represent developmental toxicity and should not be discounted as being secondary to maternal toxicity. With regard to potential teratogenic effects, Mast et al. (1992) noted that in mice that survived the exposure period, no increase was observed in the incidence of fetal abnormalities. However, an increased incidence of incomplete sternal ossification in rat fetuses was observed (DuPont Haskell Laboratory, 1980).

After consideration of all endpoints, the CNS effects and liver toxicity, observed in the subchronic NTP (1998) study, were determined to be the most sensitive effects. Furthermore, the THF database contains additional support for these endpoints from both human and animal studies (Gamer et al., 2002; BASF, 2001a; Garnier et al., 1989; Komsta et al., 1988; Horiguchi et al., 1984; DuPont Haskell Laboratory, 1979; 1980; 1996a; 1996b; Horiuchi et al., 1967; Stasenkova and Kochetkova, 1963).

The subchronic toxicity study in mice (NTP, 1998; subchronic) was selected as the principal study for the derivation of the RfC. The **RfC of 2 mg/m^3** is based on findings of CNS and liver toxicity in male mice (NTP, 1998), with a POD of 246 mg/m^3 derived from the $BMCL_{10}$ value for increased absolute liver weight. A composite UF of 100 was used. This factor is based on a default factor of 10 to account for intrahuman variability, 3 for extrapolation from an animal study for which effect levels were adjusted by appropriate animal-to-human dosimetry, and 3 to account for uncertainties in the overall inhalation toxicity database.

No sensitive subpopulations have been identified. The existing data do not provide convincing evidence for age- or gender-related differences in sensitivity to noncancer effects of THF, although there is uncertainty regarding the potential effects on postnatal development following inhalation exposure. A number of findings raise questions about the potential for increased susceptibility based on gender, including potential effects in the uterus of mice, apparent sex-specific tumor formation, and toxicokinetic differences between male and female rodents.

As noted in Section 6.1.1, a confidence level of high, medium, or low is assigned to the study used to derive the RfC, the overall database, and the RfC itself, as described in EPA (U.S. EPA, 1994), Section 4.3.9.2. The principal study used to derive the RfC (NTP, 1998) was a well-conducted and documented study reflecting high confidence. The study included subchronic and chronic exposure duration components in two species by the relevant route of exposure, evaluated a comprehensive array of tissues, and covered a well-spaced concentration range. Confidence in the supporting database is medium to high. Although chronic toxicity studies (NTP, 1998) and developmental toxicity studies (Mast et al., 1992; DuPont Haskell Laboratory, 1980) were available for the inhalation route, no multigeneration reproductive toxicity study by the inhalation route is available. Both the inhalation developmental toxicity studies (Mast et al., 1992; DuPont Haskell Laboratory, 1980) and the oral two-generation reproductive toxicity study (Hellwig et al., 2002; BASF, 1996) show that effects in fetuses and pups occur at exposures that cause at least minimal maternal effects and that these concentrations are higher than the NOAEL for organ weight changes in mice (NTP, 1998). Based on high confidence in the well-conducted principal study and medium-to-high confidence in the database, the overall **confidence in the RfC** can be characterized as **medium to high**.

6.1.3. Cancer

Under the U.S. EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), the database for THF provides “suggestive evidence of carcinogenic potential” by all routes of exposure. No epidemiological studies were identified that evaluated the carcinogenic potential of THF via the oral, inhalation, or dermal routes of exposure. A 2-year NTP (1998) inhalation cancer bioassay reported an increased incidence of hepatocellular adenomas and carcinomas in female $B6C3F_1$ mice (statistically significant trend) and an increased incidence of renal tubule adenomas and carcinomas in male F344/N rats (statistically significant trend) following inhalation exposure to 200, 600, and 1,800 ppm of THF (NTP, 1998).

The available mechanistic information and possible modes of action were evaluated for the male rat kidney tumors and female mouse liver tumors. For the rat kidney tumors, there were some data suggesting that following the inhalation exposure in the NTP (1998) bioassay, tumors developed due to the accumulation of α_{2u}-globulin. However, the data were insufficient to establish this mode of action. Also, the available evidence is not sufficient to support CPN as a potential MOA for the increase in male rat kidney tumors. For mouse liver tumors, although increased cell proliferation was noted in short-term studies, these data were not adequate to establish this mode of action. The absence of a significant increase in cell proliferation in tissues obtained from the subchronic NTP (1998) study also suggests that cell proliferation might not be a sustained response even with continued dosing. Furthermore, key precursor events linked to observed cell proliferation were not identified. The data on other potential modes of action were too limited to establish the mode of action for liver tumors induced by THF.

An IUR was not derived based on peer reviewers' concern for the potential overestimation of risk in deriving an IUR using a linear low-dose extrapolation approach combined with the uncertainty associated with the carcinogenic potential for THF. Because there may be some circumstances for which a cancer risk estimate for THF would be useful, EPA has presented, in Apprendix B, what the inhalation cancer risk estimate would be if it were derived using a linear low-dose approach. Risk assessors should use caution when considering the use of this value due to the uncertainty associated with the potential overestimation of risk related to the linear low-dose extrapolation approach employed in its derivation and the suggestive nature of the tumorigenic response.

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APPENDIX A. SUMMARY OF EXTERNAL PEER REVIEW AND PUBLIC COMMENTS AND DISPOSITION

The Toxicological Review of Tetrahydrofuran has undergone a formal external peer review performed by scientists in accordance with EPA guidance on peer review (U.S. EPA, 2006a; 2000a). The external peer reviewers were tasked with providing written answers to general questions on the overall assessment and on chemical-specific questions in areas of scientific controversy or uncertainty. A summary of significant comments made by the external reviewers and EPA's responses to these comments arranged by charge question follow. In many cases the comments of the individual reviewers have been synthesized and paraphrased in development of Appendix A. EPA also received scientific comments from the public. These comments and EPA's responses are included in a separate section of this appendix.

EXTERNAL PEER REVIEWER COMMENTS

The reviewers made several editorial suggestions to clarify specific portions of the text. These changes were incorporated in the document as appropriate and are not discussed further.

(A) GENERAL CHARGE QUESTIONS

QUESTION A1. Is the Toxicological Review logical, clear, and concise? Has EPA accurately, clearly, and objectively represented and synthesized the scientific evidence for noncancer and cancer hazard?

Comments: Generally, the reviewers regarded the Toxicological Review for Tetrahydrofuran to be comprehensive, clear, and concise. However, one of the reviewers commented that the presentation of mode of action information was repetitious, while a second reviewer suggested subheadings be added to better orient presentation of the mode of action information in Section 4.4.3. One reviewer also cited some errors and recommended clarification or changes to Section 3.3 (Metabolism) and Figure 3-1. Issues specifically related to the derivation of the oral RfD (Charge Questions B1-B6), inhalation RfC (Charge Questions C1-C6), and the cancer assessment (Charge Questions D1-D5) that were discussed by reviewers in response to Charge Question A1 are addressed under the relevant charge questions below.

EPA Response to Comments: Section 4.4.3 (now moved to Section 4.5 and Appendix C.2.2) has been revised by adding subheadings to orient presentation by topic rather than study as suggested by the reviewers. Some toxicity data unrelated to mode of action (e.g., uterine effects in mice) have been moved to Section 4.2. Section 3.3 and Figure 3.1 have been revised for clarification and additional information on THF metabolism has been included. In addition, redundant text has been removed in several sections.

QUESTION A2. Please identify any additional studies that should be considered in the assessment of the noncancer and cancer health effects of THF.

Comments: None of the reviewers provided any additional studies for consideration.

EPA Response to Comments: No response needed.

CHEMICAL-SPECIFIC CHARGE QUESTIONS
(B) ORAL REFERENCE DOSE (RfD) FOR TETRAHYDROFURAN

QUESTION B1. A chronic RfD for THF has been derived from the oral drinking water 2-generation reproductive toxicity study (BASF, 1996; Hellwig et al., 2002) in rats. Please comment on whether the selection of this study as the principal study has been scientifically justified and transparently and objectively described in the document. Please identify and provide the rationale for any other studies that should be selected as the principal study.

Comments: Five of the reviewers agreed with the selection of the Hellwig et al. (2002)/BASF (1996) study as the principal study for derivation of the chronic oral RfD. One reviewer did not provide any response. Specific issues related to this study raised by reviewers in response to this charge question are summarized and addressed under the relevant charge questions below.

EPA Response to Comments: No response necessary.

QUESTION B2. Decreased F2 male pup body weight was selected as the most appropriate critical effect. Please comment on whether the selection of this critical effect has been scientifically justified and transparently and objectively described in the document. Please provide detailed explanation. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.

Comments: Two of the reviewers agreed with the selection of decreased F2 male pup body weight gain. One of the reviewers agreed with the selection as the critical effect, but commented that decreased pup body weight gain represented a marginally adverse effect considering the possibility of lower maternal water consumption at high doses. Another reviewer commented that while the selection of the critical effect was transparently and objectively described in the Toxicological Review, this reviewer questioned whether this endpoint was appropriate to serve as a critical effect. Specifically, the reviewer stated that the effect was minimal, only observable during a small period of time, and may have been impacted by the decreased water consumption in the dams. This reviewer also stated that this effect did not lead to more pronounced changes in growth or reproductive ability in the F1 generation. However, this reviewer concluded that there did not appear to be a better endpoint from these studies that could be used to calculate the RfD. Another reviewer commented that classifying the decreased pup body weight gain as a developmental effect was tenuous due in part to the overall, low magnitude of the effect and the decreased water intake in dams. This reviewer stated that while these studies may remain the most appropriate to serve as the principal studies due to the absence of more high quality data sets, their interpretation as direct evidence of THF-induced developmental toxicity is questionable. Two reviewers noted that the supporting data were weak or minimal, while another reviewer stated that delayed eye opening was difficult to measure and thus difficult to model. One reviewer did not provide any response to this question.

EPA Response to Comments: EPA concluded that the decreases in pup body weight gain indicated delayed or altered growth following exposure to THF, an effect which is one of four major manifestations of developmental toxicity. In addition, a significant number of F2 pups/litter had delayed opening of eyes and increases in the number of sloped incisors at the high dose. These endpoints in pups are considered common markers of an adverse toxicological effect on development and are consistent with the principles and practices of the EPA's *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991a). Section 5.1.1 was augmented to further characterize the adversity of the critical effects.

There is some evidence offering support for delayed growth in the F2 generation and/or altered impact on reproductive capacity in the F1 generation. Specifically, the mean number of delivered F2 pups/litter was decreased 16% in the high-dose group compared with control and was outside the range of historical control values. The study authors considered this finding to be consistent with a slight developmental delay (Hellwig et al., 2002).

The decreased PND 7-14 pup body weight gain findings were consistent in males and females across both generations (i.e., both F1 and F2 pups) within the study, with $\geq$ 10% decreases in body weight gain as compared to control at the highest dose tested and positive trends ($p < 0.01$) in all cases. The lowest BMDL (for F1 male pups) was selected to serve as the point of departure for calculation of the RfD, following a slight correction to the time-weighted dose levels that had appeared in the external peer review draft.

With regard to comments related to maternal water intake, data on the possible relationship between decreased water intake in dams and decreased production of milk was not provided in this study. As detailed in Section 4.3.1, the decreased gain in pup body weight is supported by the statistically significant correlation between F2 pup body weight gain and maternal THF intake after multivariable regression analyses were conducted to control for the other possible confounding factors, namely average water intake and number of pups in each litter. Thus, the observed responses in the pups appear to be related to THF exposure.

QUESTION B3. The chronic RfD has been derived utilizing benchmark dose (BMD) modeling to define the point of departure (POD). All available models were fit to the individual male and female and combined incidence data (F1 and F2 pup body weight gain). Please comment on the appropriateness and scientific justification presented for individual and combined body weights to obtain a data set for BMD modeling. Please provide comments with regards to whether BMD modeling is the best approach for determining the point of departure. Has the BMD modeling been appropriately conducted and objectively and transparently described? Has the benchmark response selected for use in deriving the POD been scientifically justified and transparently and objectively described? Please identify and provide rationale for any alternative approaches (including the selection of BMR, model, etc.) for the determination of the point of departure, and if such approaches are preferred to EPA's approach.

Comments: All reviewers agreed that BMD modeling was the most appropriate approach to derive the RfD and that the F1 and F2 pup body weight gain data were suitable endpoints for deriving BMD estimates. Two reviewers included the caveat that while the BMD modeling was the most appropriate approach, the use of the pup body weight gain is a limitation (see reviewer comments for Charge Questions B1 and B2 for specifics). Four of the reviewers recommended using 1 SD below the control mean for pup body weight gain instead of a 5% decrease in body weight gain as the BMR to establish the POD, on the basis that a percentage reduction in body weight gain is an arbitrary choice compared with a measure of effect that considers the variation among animals.

EPA Response to Comments: Based on peer reviewer recommendations, the RfD for THF was derived using a BMR of 1SD. For the RfD, EPA considered BMRs of a 5% change and a 1 SD change in pup body weight gain compared with the control and developed candidate PODs using both BMRs (see Table 5-2). A 5% change in body weight gain is assumed to be relevant for pups by analogy to adult body weight, for which a 10% change is generally recognized to support identification of maximum tolerated doses. A 1 SD BMR considers the variation among animals, through the inference of a normal range based on the control group. In the case of decreasing weight gains, a 1 SD change from the control mean corresponds to 10% of an exposed population having larger decreases in body weight gain than the lowest 1% of the control group, when the data follow a normal distribution (U.S. EPA, 2000b). In consideration of the peer reviewer recommendations, EPA selected a POD associated with a BMR of 1SD for the derivation of the RfD.

The external peer review draft included results based on the SDs reported by the investigators; these SDs characterized the variability between litter means of pup body weights. However, it is the variability among pups which is relevant in order to use a 1SD BMR, not the variability between litters. EPA developed the relevant SD estimates (from the Hellwig et al., 2002/BASF, 1996 study) and incorporated these estimates in the BMD modeling analysis for the RfD. Section 5 and Appendix B of the Toxicological Review were revised to reflect the use of a BMR of 1SD for RfD derivation.

QUESTION B4. Please comment on the selection of the uncertainty factors applied to the POD for the derivation of the RfD. For instance, are they scientifically justified and transparently and objectively described in the document?

Comments: Three of the reviewers agreed with the selection of the UFs applied to the POD for the derivation of the RfD, and two of these reviewers further noted that the selections were scientifically justified and clearly described. One reviewer did not provide comments on the selection of UFs. One reviewer stated that the overall UF was not warranted and indicated that the Toxicological Review presents data suggesting that metabolism of THF does not have a role in THF-induced toxicity (i.e., metabolism is not a rate-limiting step) and that there may not be a 10-fold variability among individuals or among species. This reviewer, therefore, was of the opinion that each of the intra- and interspecies UFs should be no more than 3. This same reviewer also suggested a reduction in the database UF because the RfD was based on a very sensitive endpoint from a well-conducted study. Another reviewer stated the selection of uncertainty factors was transparent and objectively described but questioned the composite UF, stating that the oral toxicity was so low it was difficult to find an endpoint of concern. This reviewer noted that the interspecies UF could be reduced based on the fact that the water solubility of THF would make it unlikely that THF would be absorbed and distributed differently in rodents compared to humans, and by using the inhalation toxicity database to inform the oral toxicity database the database UF could be reduced.

EPA Response to Comments: In response to the comments from two reviewers who questioned the selection of UFs for the RfD, EPA re-evaluated the rationale for each of the UFs and revised text for clarification. With regard to the role of metabolism in THF-induced toxicity, the data are unclear. There is some evidence that one of the THF metabolites, GHB is linked with the neurotransmitter GABA and CNS effects (i.e., one of the critical effects for the RfC); indicating metabolism may play a role in THF-induced toxicity (see Section 3.3). Additionally, while there is no information on either the toxicokinetics of THF following exposure by the oral route or on differential sensitivity of human populations to THF, blood kinetics data were highly variable among volunteers exposed to THF by the inhalation route (Kageyama, 1988 covered in Section 3.1.2). Moreover, there is potential for variation in humans and across species. The metabolism section (Section 3.3) has been revised to include literature on THF metabolism and on the role that lactonase (also known as PON1) may play in hydrolyzing GBL (a lactone intermediate) to GHB. There is a wide inter-individual variation in PON1 concentration and activity of up to 13-fold (van Himbergen et al., 2006) and possibly in some CYP450 isoenzymes, which may be involved in the early steps of oxidative metabolism of THF to GBL. It is not clear if, and to what extent, genetic variability in expression and activity of PON1 and CYP450 may influence the kinetics of THF biotransformation, and how, in turn, such variability might influence human risk to THF exposure (see Section 4.8.3). Furthermore, no information is available on life-stage susceptibility to THF exposure. Therefore, the default value of 10 for UF_H was applied.

With respect to interspecies variability, there are some limited data by the inhalation route of exposure suggesting qualitative toxicokinetic similarities between humans (Droz et al., 1999; Ong et al., 1991; Kageyama, 1988) and rats (Elovaara et al., 1984; Kawata and Ito, 1984). For instance, THF was rapidly excreted following repeated inhalation exposure in both species with limited bioaccumulation (see Section 3). However, these data are not adequate to provide a quantitative estimate of toxicokinetic differences. Also, the human inhalation exposure PBPK model for estimating THF concentration in blood, breath, and urine (Droz et al., 1999) does not account for the toxicokinetic variability in humans, and no similar PBPK model has been identified in animals (see Section 3.6). Furthermore, there are no comparative toxicokinetic or toxicodynamic studies following exposure to THF by the oral route in humans and animals. Thus, the default UF_A of 10 was applied to account for interspecies differences.

Regarding the comment on the composite UF in light of THF's toxicity, it is Agency practice to apply UFs of 1, 3 (i.e., $10^{1/2}$ = 3.16 rounded to 3), or 10 (U.S. EPA, 2002) for five areas of uncertainty to a selected POD as representative of a chemical's toxicity. In the case of THF, factors of 10 were applied for intraspecies and interspecies extrapolation and to account for database deficiencies resulting in a composite UF of 1000. The Agency considered these UFs to be warranted as there are several data gaps, described above briefly and in further detail in Section 5.1.3, that support the application of 10-fold factors.

The comments on the database UF are addressed below in response to comments to Charge Question B6.

QUESTION B5. A two-generation reproductive toxicity study was used for the selection of the POD for the derivation of the RfD. Please comment on whether the rationale and justification for not applying a subchronic to chronic uncertainty factor has been scientifically justified and transparently described in the document.

Comments: Five of the reviewers agreed with the rationale and justification for not applying a subchronic to chronic UF. One reviewer did not provide comments.

EPA Response to Comments: No response needed.

QUESTION B6. Please comment on whether the rationale and justification for the selection of the database uncertainty factor has been scientifically justified and transparently described in the document.

Comments: Three reviewers agreed with the selection of the database UF and two of these further stated that the rationale and justification for this selection was scientifically justified and transparently described. One reviewer agreed that the selection of the database UF was transparent and objectively described, but commented that the selection may need better justification. This reviewer considered the overall oral toxicity of THF to be low and that both the oral and inhalation toxicity data were adequate for estimating chronic toxicity for THF, thus reducing the need for the UF_D. Another reviewer stated that the selection of the database UF was not justified considering the principal study was well-documented and the RfD was based on a very sensitive endpoint. One reviewer did not provide any response.

EPA Response to Comments: In response to the comment from the reviewer who suggested utilizing both the oral and inhalation databases to inform the selection of the oral database UF, the rationale for the UF was re-examined. Considering the limited oral database (only a well-conducted two-generation reproductive study with limited tissue histopathology and a one-generation study that did not include a histopathological examination of tissues), the Agency attempted to utilize the inhalation database by evaluating the application of a route-to-route extrapolation approach (see Sections 3.6, 5.1.1, and 5.1.3). However, due to inadequate PBPK models and the absence of absorption efficiency data in humans and rats, this approach was not scientifically supported. Another alternative approach involving the use of the oral data for metabolites of THF for deriving the RfD was considered (described in Section 5.1.1), but because there is uncertainty surrounding the role of metabolism in THF-induced toxicity, this approach was not adopted. Although these alternative approaches were inadequate, the Agency did consider the information associated with inhalation exposure. The inhalation database lacks a two-generation reproductive toxicity study but contains developmental, chronic, and subchronic toxicity studies in rats and mice (NTP, 1998; Mast et al., 1992; DuPont Haskell Laboratory, 1980). The inhalation developmental toxicity studies provided evidence of effects on the fetus. The subchronic and chronic studies reported systemic toxicity (CNS effects and liver weight changes) at exposure concentrations lower than those inducing developmental toxicity; suggesting that systemic toxicity may be of more concern. Thus, the lack of studies examining endpoints other than reproductive toxicity following oral exposure is a database deficiency, and a 10-fold UF was applied. The text in Section 5.1.3 has been augmented to provide a more clear description of the available database and identification of the deficiencies.

(C) INHALATION REFERENCE CONCENTRATION (RfC) FOR TETRAHYDROFURAN

QUESTION C1. A chronic RfC for THF has been derived from data from a 105 week chronic inhalation study (NTP, 1998) in mice and rats. Please comment on whether the selection of this study as the principal study has been scientifically justified and transparently and objectively described in the document. Please identify and provide the rationale for any other studies that should be selected as the principal study.

Comments: All reviewers stated that they were supportive of EPA's selection of the 105-week chronic inhalation study as the principal study to derive the RfC for THF.

EPA Response to Comments: Charge Question C1 inaccurately states that the RfC for THF was derived based on data from a 105 week chronic inhalation study (NTP, 1998) in mice and rats. The RfC derived in the external peer review draft was based on the 14-week subchronic NTP (1998) study which identified both CNS and liver effects in mice. Both the 14-week and 105-week studies are reported as NTP (1998).

The external peer review draft stated that based on clinical signs of CNS toxicity and liver effects, a NOAEL of 1,770 mg/m^3 and a LOAEL of 5,310 mg/m^3 were identified. CNS effects were observed at 5,310 mg/m^3 and 14,750 mg/m^3 in the subchronic study, and at 5,310 mg/m^3 in the chronic study. The draft also noted that THF induced a concentration-dependent increase in liver weight in male and female mice and rats and centrilobular cytomegaly in male and female mice in the subchronic study. The chronic study evaluated body weight and clinical signs of toxicity; organs were subjected to histopathological examination at necropsy. However, no measurements were taken for organ weights, hematology, or clinical chemistry in the chronic study. In addition, the chronic study did not identify liver cytomegaly in any of the exposure groups, although a slight increase in necrosis was observed in the livers of the 5,310 mg/m^3 female mice. Thus, the liver weights and histopathology (cytomegaly) cited in the discussion of the selection of the principal study and critical effect in the external peer review draft were those observed in the subchronic component of the NTP (1998) study. In light of the potential for confusion between the subchronic and chronic studies, the document has been revised to further clarify the effects reported by each study component (subchronic versus chronic exposure duration) from NTP (1998) and the rationale for the selection of the principal study. Additionally, Table 5-3 was added to more clearly characterize the two phases of the NTP study.

QUESTION C2. Liver toxicity and CNS effects were selected as the co-critical toxicological effects. Please comment on whether the selection of this critical effect has been scientifically justified and transparently and objectively described in the document. Specifically, please address whether the selection of liver effects and CNS toxicity as the co-critical effects instead of increased thymus weight has been adequately and transparently described. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.

Comments: Four of the reviewers agreed with the selection of liver toxicity and CNS effects as co-critical effects. Two of these reviewers stated that the liver effects were minimally adverse, but appropriate to select nonetheless. One of these two reviewers attributed less toxicological significance to the liver effects than to the CNS effect, while the second reviewer expressed preference for using cytomegaly over increased liver weight as a liver toxicity endpoint. One reviewer supported the use of CNS effects as the critical effect, but disagreed with the designation of liver toxicity as a co-critical effect stating that observations of hepatocytomegaly, without well characterized events such as sustained cell proliferation or decreased apoptosis, is a questionable critical effect. This reviewer stated that while cytomegaly is consistently reported, this effect is readily reversible and may be regarded as a less serious toxic endpoint; and concluded that the changes in liver weight and cytomegaly observed following THF exposure and the lack of additional key events in the liver collectively support the decision to not select liver changes as the critical effect and suggested that the assessment would benefit substantially by selecting the CNS effects alone as the critical effect for the derivation of the RfC. One reviewer did not provide comment.

Five of the reviewers agreed that thymus weight should not be used as a critical effect since it was not accompanied by either histopathological changes or measured alterations in immune competence. One reviewer did not provide comment. No other endpoints were identified by the reviewers as effects that should be considered in the selection of the critical effect.

EPA Response to Comments: EPA agrees with the reviewers who indicated that the CNS and liver effects were appropriate for use as the co-critical effects. In addition, Section 5.2.1 has been augmented to include additional discussion of liver and CNS findings. In the subchronic NTP (1998) study, liver weights (both absolute and relative) were increased in the 14,750 mg/m^3 female rats and this finding was accompanied by increased serum bile acid concentration in the absence of cholestasis or hepatocellular necrosis. The study authors indicated that these changes were consistent with decreased or altered hepatic function. In male mice, absolute and relative liver weights were statistically significantly increased following exposure to concentrations of ≥1,770 mg/m^3. The increases in absolute and relative liver weights in male mice were supported by increased incidence of centrilobular cytomegaly, statistically significant at 14,750 mg/m^3 (7/10 compared to 0/10 in the control group). Also, relative and absolute liver weights were statistically significantly increased in female mice beginning at 5,310 mg/m^3 and were accompanied by centrilobular cytomegaly (10/10 animals compared to 0/10 in controls) at 14,750 mg/m^3. The hepatocytes were additionally described as having slight karyomegaly (enlarged nucleus), increased cytoplasmic volume, and granular cytoplasm with less vacuolation than that of midzonal and periportal hepatocytes (NTP, 1998). No clinical chemistry measurements were performed in mice. The study authors concluded that the histopathological changes observed in the high exposure male and female mice groups suggested that the liver is the target organ for toxicity. They also stated that the liver weight increase and mild histopathological changes observed at the lower THF exposure concentration (5,310 mg/m^3) were consistent with a treatment related effect (NTP, 1998). Furthermore, in the chronic study liver necrosis was noted in female mice of the 5,310 mg/m^3 exposure group. The toxicological significance of the observed liver weight changes was considered to be uncertain at the low concentrations where the changes were of minimal severity and were not accompanied by other signs of liver toxicity. However, the increases in absolute and relative liver weights at 5,310 mg/m^3 were greater than 10% above controls (statistically significant) and were accompanied by minimal increases in histopathology findings (1/10 incidence in centrilobular cytomegaly) that progressed with increases in THF concentration. The liver effects observed at the exposure concentrations ≥ 5,310 mg/m^3 were therefore considered biologically significant and representative of adverse effects (U.S. EPA, 2002). Section 5.2.1 was revised to better characterize and support the selected critical effects and their toxicological significance.

Considering the information described in Section 5.2.1 (briefly described above) as well as the supporting data in acute and short-term studies (described in Appendix C), EPA concluded that liver effects and CNS effects are appropriate as co-critical effects for derivation of the RfC. Section 5.2.3 includes the candidate PODs associated with these effects as well as the potential RfCs (which are similar) for the liver and CNS effects.

EPA agrees with the reviewers regarding thymus weight as inappropriate for use as a critical effect for the derivation of the RfC.

QUESTION C3. The chronic RfC has been derived utilizing benchmark dose modeling to define the point of departure (based on liver cytomegaly). BMD modeling was conducted on liver weight and cytomegaly data in both males and females. Has the BMD modeling been appropriately conducted and objectively and transparently described? Has the benchmark response selected for use in deriving the POD been scientifically justified and transparently and objectively described? Please provide comments on whether the selection of a POD based on liver cytomegaly instead of liver weight is scientifically justified and transparently described. Please identify and provide rationale for any alternative approaches (including the selection of BMR, model, etc.) for the determination of the point of departure, and if such approaches are preferred to EPA's approach.

Comments: All of the reviewers commented that the BMD modeling was appropriate, and generally noted that the choices were objectively described and scientifically justified. However, one reviewer questioned the selection of liver cytomegaly rather than the liver weight modeling results to define the POD for deriving the RfC. This reviewer stated that given the choice between cytomegaly and increased liver weight data, the liver weight data may be a more appropriate endpoint to model, but stated that liver weight changes not accompanied by cell proliferation and/or apoptosis may not be representative of toxicity. This reviewer suggested that the POD should be based on the CNS effects. In addition, one reviewer thought it was unclear why only the male mouse data was modeled instead of the female mouse data or both sexes combined. Two reviewers suggested expanding the explanation in Appendix B of the Akaike Information Criterion (AIC).

EPA Response to Comments: Further consideration of the BMD modeling and NOAEL/LOAEL approaches, described in Section 5.2.2, provides evidence of similar PODs (as BMDLs) for both hepatocytomegaly and increased liver weight in the male mice as well as the POD (as a NOAEL) for CNS effects. Sections 5.2.1 and 5.2.2 were revised to further discuss the toxicological significance of the liver and CNS endpoints and to better characterize the modeling and candidate PODs. The discussion of the selection of the POD for derivation of the RfC was expanded in Section 5.2.2. Section 5.2.3 includes RfCs for both liver and CNS effects. The selection of the male mouse data was based on the fact that the males were slightly more sensitive (i.e., by about one dose-spacing unit) than females and text was added to Section 5.2.2 to clarify this selection. Additional text has been added to Section 5.1.2 and Appendix B describing the AIC.

QUESTION C4. No incidence data were presented for CNS effects. Thus, these data could not be evaluated by BMD modeling. However, a NOAEL-LOAEL approach (based on the CNS data) for the derivation of the RfC has been presented for comparison purposes. Please provide comments as to whether the NOAEL-LOAEL approach based on the POD for CNS effects is more appropriate for the derivation of the RfC. Please provide comments with regards to whether BMD modeling is the best approach for determining the point of departure.

Comments: Two reviewers considered the CNS effects as having greater toxicological significance than the hepatic effects and therefore supported the use of a NOAEL/LOAEL approach to derive the RfC. Two of the reviewers commented that the NOAEL/LOAEL and BMD modeling approaches yielded the same results and had a preference for the use of BMD modeling. One reviewer agreed with the approach, analysis, and discussion and conclusions presented in the Toxicological Review. One reviewer stated that both approaches were appropriate and that confidence was increased by the fact that the approaches provided the same value.

EPA Response to Comments: See responses to comments under Questions C2 and C3.

QUESTION C5. Please comment on whether the selection of the uncertainty factors applied to the POD for the derivation of the RfCs. For instance, are they scientifically justified and transparently and objectively described in the document.

Comments: Two reviewers agreed with the selection of the uncertainty factors applied to the POD for the derivation of the RfCs and noted the selection was scientifically supported and clearly described. A third reviewer agreed with the selection of the intraspecies UF (UF_H) but commented that the UF for interspecies differences ($UF_A = 3$) should not be reduced. Specifically, this reviewer disagreed with the use of a default value of 1 as the interspecies dosimetric adjustment factor as a substitute for the interspecies UF; both of which he considered as placeholders for lack of information. This reviewer recommended that the UF not be reduced until it can be replaced with a data-driven UF based on a physiologically-based pharmacokinetic model. Conversely, another reviewer suggested that both the inter- and intraspecies UFs could be reduced based on the consideration that metabolism of THF does not have a role in THF-induced toxicity (i.e., metabolism is not a rate-limiting step); thus, there may not be a 10-fold variability among individuals or among species. This reviewer also stated that the inhalation database was adequate and that the available data were better documented than the oral database. Another reviewer commented that the total UF may be overly conservative and that additional discussion should be added to the document to further explain the selection of the uncertainty factors. One reviewer provided no response to this question.

EPA Response to Comments: With regard to the reviewer comment on the 3-fold interspecies UF, when the Agency derives inhalation reference concentrations, the point of departure is adjusted for continuous exposure then converted to a human equivalent concentration (HEC) in accordance with EPA's *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry Methodology* (U.S. EPA, 1994b). In cases where there are either no data available or where the animal blood-air partition coefficient is larger than the human coefficient, a default value of 1 is used. In this instance, due to the lack of information regarding the ratio of human to animal blood-air partition coefficients for THF this ratio is assumed to be 1. With regard to the UF, the interspecies UF is comprised of two areas of uncertainty (i.e., toxicokinetic and toxicodynamic uncertainties). The determination of an HEC as described in the RfC methodology (U.S. EPA, 1994b) mostly addresses the toxicokinetic component and a 3-fold UF is applied to account for the remaining uncertainty regarding the toxicodynamic differences between mice and humans. This approach is further described in Section 5.2.2.1 and in EPA's Inhalation Dosimetry Methodology cited above.

The comments provided by one reviewer regarding reducing the intra- and interspecies UFs based on metabolism data were similar to those provided for the UFs for the oral RfD; see response to these comments under Charge Question B4. In response to the reviewer that suggested the UFs in combination may be overly conservative, EPA re-evaluated the selection of the uncertainty factors. As noted in response to comments under Charge Question B4, it is Agency practice to apply UFs of 1, 3 ($10^{1/2}$ = 3.16 rounded to 3), or 10 (U.S. EPA, 2002) for five areas of uncertainty. In the case of inhalation database for THF, a relatively low composite uncertainty factor of 100 (comprised of a factor of 10 for intraspecies variability in the absence of information regarding human variability, and factors of 3 for toxicodynamics uncertainties in extrapolating from animals to humans and for a database lacking a multigeneration reproductive toxicity study by the inhalation route of exposure) was applied. The Agency concluded that further reductions in these UFs were not justified. See Section 5.2.3 for description of UFs. The comment related to the database UF is addressed in response to comments under Charge Question C6 specific to the database UF.

QUESTION C6. Please comment on the transparency and scientific rationale and justification for the selection of the database uncertainty factor. Please comment on whether the application of the database uncertainty factor adequately represents the gap in inhalation reproductive and developmental toxicity and immunotoxicity data for THF. Please comment on whether the rationale for use of the oral data to inform this decision scientifically justifiable and transparently described in the document.

Comments: Four of the reviewers agreed with the selection of the database UF of 3. One of these reviewers stated that the explanation was transparent and another noted it was clear and well explained. One reviewer did not provide any response to this question. Two reviewers specifically commented on the immunotoxicity data. One of these reviewers stated that immunotoxicity may not be cause for concern with respect to the database UF, as there was no evidence to indicate that lymphocyte cell populations would be selectively sensitive to THF. In addition, this reviewer noted that cytotoxicity was not demonstrated in the available mode of action studies for THF. This reviewer further suggested that due to the rapid metabolism of THF, there was less concern for immunotoxicity at chronic low exposures to THF and that secondary effects that may result from inflammatory responses produced at high exposures would not be relevant to low exposures. Another reviewer agreed that the lack of immunotoxicity data should not be a concern, and noted that a 3-fold database UF may not be needed.

EPA Response to Comments: EPA agreed with the reviewers' comments in response to Charge Questions C2 and C6 regarding immunotoxicity data and has revised the Toxicological Review to indicate that thymus effects observed following exposure to THF are not likely to represent a specific concern. Additionally, the rationale for the database UF in Section 5.2.3 was revised to remove immunotoxicity as an uncertainty in the inhalation database for THF. An uncertainty factor of 3 was applied to account for the lack of a two-generation reproductive study.

QUESTION C7. THF induces a spectrum of effects consistent with both Category 1 and Category 3 gases. Therefore, for the purposes of calculating HECs, respiratory tract effect levels were calculated using the default equations for Category 1 gases and extrarespiratory tract effect levels were calculated using default equations for Category 3 gases. Please comment on the explanation for the dosimetry choice in the derivation of the RfC. Has the rationale been scientifically justified and transparently described?

Comments: Five reviewers agreed with EPA's dosimetry choices. One reviewer did not respond. Two reviewers that commented noted that this section could be improved by additional discussion of the gas categories, and a third stated that he was unfamiliar with EPA's categorization of gases, suggesting additional discussion of these categories would be beneficial.

EPA Response to Comments: EPA agrees with the reviewers regarding the dosimetry choices and Section 5.2.2.1 was revised to better characterize the approach used to calculate HECs for the endpoints considered as the basis for the RfC as described under the EPA's *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b). Detailed classification information for Category 1, 2, and 3 gases is also provided in the cited EPA report (U.S. EPA 1994b).

(D) CARCINOGENICITY OF TETRAHYDROFURAN

QUESTION D1. Under the EPA's 2005 Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005), there is suggestive evidence for the human carcinogenic potential of THF. Please comment on the scientific justification for the cancer weight of the evidence characterization. A quantitative cancer assessment has been derived for THF. Do the data support estimation of a cancer slope factor for THF? Please comment on the scientific justification for deriving a quantitative cancer assessment considering the uncertainty in the data and the suggestive nature of the weight of the evidence of carcinogenic potential.

Has the rationale and scientific justification for quantitation been transparently and objectively described?

Comments: Five reviewers agreed with the "suggestive evidence of carcinogenic potential" cancer descriptor. One reviewer did not comment. Two reviewers commented that, while the evidence for female mouse liver tumors can support quantitative estimation of cancer potency, the dose-response of the male rat kidney tumor data was weak. Two reviewers commented that a quantitative assessment would overestimate the risk due to their opinion that THF is a very weak possible human carcinogen. Another reviewer noted that the quantitative cancer assessment may provide a measure of the magnitude of the carcinogenic concern. Several reviewers commented on the quantitative impact of the linear low-dose extrapolation approach utilized in deriving a cancer risk estimate; these comments are address under Charge Question D4.

EPA Response to Comments: EPA agrees with the consensus view regarding the cancer descriptor. Based on the peer reviewers' concern for the potential overestimation of risk in deriving an IUR for THF using a linear low-dose extrapolation approach, and the uncertainty associated with the carcinogenic potential for THF, EPA did not derive an IUR. Please see EPA's response to the issues related to the quantitative estimate of risk under Charge Question D4.

QUESTION D2. The available data suggest that a plausible mode of action for THF-induced male rat kidney tumors may involve the accumulation of alpha-2u globulin. EPA concluded that the available data do not provide significant biological support to establish a mode of action for male rat kidney tumors and that these tumors are relevant to humans. Please comment on the transparency and scientific rationale and justification for the evaluation of these data and the conclusions regarding the possible mode(s) of action and human relevance for the male rat kidney tumors.

Comments: Four reviewers agreed with the conclusions that the available data do not provide significant biological support to establish a mode of action for male rat kidney tumors and that these tumors are relevant to humans. Two reviewers did not comment.

EPA Response to Comments: No response needed.

QUESTION D3. The available data suggest that increased proliferation and promotion in the liver may be a plausible mode of action for THF-induced female mouse liver tumors.

EPA concluded that the data do not provide significant biological support to establish a mode of action for female mouse liver tumors and that these tumors are relevant to humans. Please comment on the transparency and scientific rationale and justification for the evaluation of these data and the conclusions regarding the possible mode(s) of action and human relevance for the female mouse liver tumors.

Comments: Three of the reviewers agreed with the conclusion that the available data do not provide significant biological support to establish a mode of action for female mouse liver tumors and that the liver tumors are relevant to humans. One reviewer agreed that the mode of action for THF-induced liver tumors is unknown, but suggested that chronic irritation (which this reviewer considered as the most plausible mechanism of action for very low potency cancer-causing chemicals) may be the mode of action of THF-induced liver tumors. The reviewer also commented that the female mouse liver tumors were not relevant to humans because of the lack of sufficiently high exposures and the very low incidence of liver cancer in humans compared to $B6C3F_1$ mice. Another reviewer's comments on the extrapolation approach are summarized and addressed under Charge Question D4. One reviewer provided no response to this question.

EPA Response to Comments: As described in Sections 4.7.1 and 5.3.6, though it is possible that THF may act as a tumor promoter, there is no information on potential precursor or key events, and the possible role of chronic inflammation or specific mediators of tumorigenesis for THF has not been examined. Thus, EPA concluded that in the absence of mode of action information the mouse liver tumors were relevant.

QUESTION D4. An inhalation unit risk has been derived utilizing benchmark dose modeling to define the point of departure of 10% extra risk followed by linear low-dose extrapolation below the point of departure (i.e., the default assumption). Please comment on the scientific justification and rationale supporting the estimation of an inhalation unit risk from the available data for THF. Specifically, please comment on whether the rationale for the quantitative analysis is objectively and transparently described, considering the uncertainty in the data and the suggestive nature of the weight of evidence. Please comment on the selection of linear low dose extrapolation. Has the justification of linear low dose extrapolation been objectively and transparently presented? Please identify and provide rationale for any alternative approaches for low dose extrapolation that the data for THF would support and if such approaches are preferred to EPA's approach.

Comments: One reviewer agreed with the selection of the linear extrapolation approach in the absence of data to support other options. Two reviewers commented that the discussion of the decisions leading to the derivation of the inhalation unit risk and the decision to select a linear low dose extrapolation was objectively and transparently described. Four reviewers disagreed with the selection of a linear low dose extrapolation based on the following reasons: THF is not genotoxic/DNA-reactive, its metabolism is rapid and doesn't form a reactive metabolite, it doesn't cause irreversible damage, it induced a weak tumor response at high doses, and it doesn't induce proliferative lesions considered to be pre-neoplastic; concluding that the application of a linear low-dose extrapolation approach will overestimate cancer risk. One reviewer also noted that all of the biological effects identified for THF are those which are commonly thought to exhibit thresholds; this reviewer and another reviewer recommended using a reference value approach to estimate a non-carcinogenic dose. Another reviewer proposed survival adjustments to the tumor incidence rates that should be considered in the dose-response modeling and derivation of the inhalation unit risk. This reviewer presented several choices to consider for selecting dose-response models, and included a nonlinear approach in response to Charge Question D3. One reviewer provided no direct response to this question (this reviewer provided relevant comments under other Charge Questions; these comments are incorporated above). The reviewers who recommended a nonlinear approach suggested that a nongenotoxic carcinogen would accordingly have a nonlinear cancer response at low dose.

EPA Response to Comments: As described in Section 5.3.1, based on the peer reviewers' concern for the potential overestimation of risk in deriving an IUR for THF using a linear low-dose extrapolation approach, and the uncertainty associated with the carcinogenic potential for THF, EPA did not derive an IUR. Sections 5.3, 6.1.3, and Appendix B were revised accordingly and text was added to transparently describe the uncertainties and the opinions of the peer reviewers.

As noted in the Cancer Guidelines, there are some circumstances (e.g., providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities) in which a cancer risk estimate for THF would be useful (U.S. EPA, 2005a). Thus, EPA presented a dose-response analysis (see Appendix B) to show what the inhalation cancer risk estimate would be if derived using a linear low-dose extrapolation approach. In considering the reviewer comments on the dose-response analysis, EPA agrees that the differential survival among the female mice and male rats, as noted by one reviewer, should be reflected in any cancer risk estimates, and has revised the dose-response data accordingly (See Table B-3).

Additionally, EPA considered the model selection options proposed by one reviewer, which included using a multistage approach as presented in the external peer review draft

Toxicological Review of Tetrahydrofuran. As noted by this reviewer, the multistage model is supported by biological plausibility through its parallelism to the multistage carcinogenic process, and has been widely used for cancer risk assessments. In cases such as THF, where there is no biologically based model available and insufficient support for considering nonlinear low-dose extrapolation, EPA uses the multistage model, which also maintains consistency across cancer assessments, unless the multistage model does not fit the observed dose-response. In this case, a one-stage multistage model provided a more parsimonious fit of the survival-adjusted data than the fit (three-stage multistage) offered by the reviewer.

EPA notes that the suggestions for simple or weighted BMDL averaging both produce PODs that are not well-defined confidence limits. That is, for component BMDLs that are 95% confidence limits, neither type of average leads to a 95% confidence limit for the combined result, and the level of confidence is not easily determined. More comprehensive analysis involving model averaging is a significant area of research currently, but scientific consensus has not yet been reached regarding how to implement model averaging, including which and how many models to consider.

To address the reviewers' comments regarding the inclusion of a nonlinear approach, text was added to Section 5.3.1 and Appendix B to expand the discussion of extrapolation approaches. For THF, there are no data to inform the shape of the dose-response curve at low doses and there are no noncancer effects reported that could serve as a precursor endpoint upon which to base a nonlinear analysis. Thus, the nonlinear analysis recommended by the peer reviewers cannot be readily implemented. One of these reviewers also suggested carrying out a nonlinear low-dose extrapolation by using a $BMCL_{10}$ estimated from modeling the tumor data and applying uncertainty factors for human variability, extrapolation from animals to humans, and for database uncertainty. Because the mode of carcinogenic action is not known (as noted by EPA and the peer reviewers) and the use of a key event to represent a POD for a threshold approach could not be implemented, along with the uncertainty associated with the suggestive nature of the carcinogenic potential, EPA did not explore this option further and chose instead to not derive an IUR for THF.

QUESTION D5. THF induces a spectrum of effects consistent with both Category 1 and Category 3 gases. Therefore, for the purposes of calculating human equivalent concentrations, respiratory tract effect levels were calculated using the default equations for Category 1 gases and extrarespiratory tract effect levels were calculated using default equations for Category 3 gases. Please comment on the explanation for the dosimetry choice in the derivation of the inhalation unit risk. Has the rationale been scientifically justified and transparently described?

Comments: Three reviewers agreed with the HEC calculations. Three reviewers did not comment in response to this question.

EPA Response to Comments: No response needed.

PUBLIC COMMENTS

Comments: A commenter stated that the critical endpoint (decreased weight in F2 pups) for deriving the RfD is weak and equivocal because the findings may have been due to reduced maternal milk production associated with decreased water intake and/or food consumption. The commenter also questioned the rationale behind the gender-specific effect on weight gain in male, but not female, pups and expressed doubts about a direct association between this reduced weight gain in male pups and THF exposures.

EPA Response to Comments: See response to charge question B2 in Appendix A. There are no data evaluating the possible relationship between decreased water intake in dams and decreased production of milk (i.e., milk output was not measured). However, after multivariable regression analyses were conducted to control for possible confounding factors, including water intake and number of pups in each litter, it was found that the decreased in F2 pup body weight gain was statistically significantly correlated with maternal THF intake. Therefore, decreased pup body weight gain is considered an appropriate endpoint for deriving the RfD. The study authors concluded that the decreased pup body weight gain could be a high concentration effect reflecting general toxicity due to direct exposure to THF during lactation (Hellwig et al., 2002). Specifically, the study authors suggested that, given that THF is slightly more soluble in lipid than water, THF may have been more concentrated in the dam's milk fat than in the maternal water compartment. Based on the developmental effects observed (decreased pup body weight gain, delayed eye opening, and increased incidence of sloped incisors) the study authors designated 3,000 ppm as the NOAEL. Maternal food consumption was marginally decreased in F0 and F1 lactating dams and the decrease was not associated with statistically significant reduction in maternal body weight gain. Finally, there is no apparent gender-specific effect on weight gain in both the F1 and F2 pups. While for F1 pups, the responses at each dose were different between males and females, for F2 pups, the responses were comparable between males and females at all doses. It is not clear if there is sex dependence for effects on F1 but not F2 pups or if there is a biological basis or this difference reflects only statistical considerations.

However, the BMD and BMDL estimates for the combined F2 data were similar to the BMD and BMDL estimates derived for either sex individually. Therefore, EPA concluded that data corresponding to the F2 females, described by the linear model, provided the best fit and the corresponding $BMDL_5$ value of 303 mg/kg-day was used to derive the RfD.

Comments: A commenter stated that the composite UF (1,000) used to derive the RfD for THF is excessive given the apparent toxicity of THF. Specifically, the commenter stated that an UF of 10 to account for deficiencies in the oral database exaggerates the potential significance of limitations in the oral data.

EPA Response to Comments: See response to Charge Question B4. As noted in Section 5.1.3, an UF_D of 10 was selected to account for deficiencies in the toxicity database for oral exposure to THF. The oral database for THF contains a two-generation reproductive toxicity study and a range-finding one-generation reproductive study (Hellwig et al., 2002; BASF, 1996, 1994). There are no available human occupational or epidemiological studies or standard toxicity studies, including developmental toxicity studies, in animals.

Comments: A commenter questioned the use of CNS depression as one of the critical endpoints to derive the RfC because transient sedation from exposure to this volatile organic chemical is reversible and does not by itself provide any evidence of sustained neurotoxicity.

EPA Response to Comments: EPA agreed with the peer reviewers' comments supporting the use of CNS effects as a co-critical effect for the derivation of the RfC. Text was added to Section 5.2.1 to further discuss significance of these effects and the rationale for the selection.

Comments: A commenter recommended using a combined UF of 30 rather than 100 to derive the RfC for THF. The commenter stated that the inhalation database for THF is relatively robust obviating the need for a database UF of 3. Among the reasons that were cited in support of this view were that adult animals were more sensitive than fetuses or weanling animals, the offspring findings in the oral two-generation study were unremarkable, and the absence of other immunotoxicity findings (such as histopathology) that may lend support to the effect on thymus weight.

EPA Response to Comments: Based on comments from the external peer reviewers, EPA has revised the text to indicate that thymus effects observed following exposure to THF may not represent an uncertainty in the database. An uncertainty factor of 3 was selected to account for deficiencies in the database for THF. Chronic and subchronic inhalation bioassays and developmental toxicity studies are available in rats and mice (NTP, 1998; Mast et al., 1992; DuPont Haskell Laboratory, 1980). No two-generation reproductive toxicity study by the inhalation route is available.

Comments: A commenter stated that the carcinogenicity data for THF, particularly the liver tumor response in female mice, support at most a classification of suggestive evidence of carcinogenic potential in humans. The commenter disagreed with EPA's determinations regarding the mode of action for carcinogenicity. The commenter agreed with EPA's conclusion that the currently available data do not clearly establish α_{2u}-globulin accumulation as mode of action for kidney effects. The commenter suggested, however, that renal tumors may have been related to THF-induced exacerbation of or interaction with CPN (Hard, 2005). The commenter asserted that evidence suggests that CPN may not be relevant to humans (Hard and Khan, 2004) and that there is a causal link between CPN, atypical tubule hyperplasia (ATH), and adenomas in rats. The commenter stated that there is sufficient evidence to clearly establish CPN as the mode of action for increased incidence of renal tumors in male rats based on two evaluations by the Tetrahydrofuran Task Force (Fenner-Crisp, 2007; Hard, 2005). The commenter also cited a recent NTP publication that evaluated α_{2u}-globulin-associated nephropathy and renal tumors in rats (Doi et al., 2007) as support for the conclusion that there is a causal link between CPN and proliferative lesions in the kidney.

EPA Response to Comments: EPA agreed with the peer reviewers' comments that the mode of carcinogenic action for THF has not been established. Hard (2005) concluded that in the chronically exposed control and high exposure male rat groups there were comparable incidences of ATH (5/50 and 6/50, respectively). In addition, Hard (2005) reported that the treated male and female group incidences and severity of CPN were almost identical to the respective male and female control groups.

There was no difference in the incidence or severity of CPN in male rats of the NTP 2-year carcinogenicity study on THF (both the control and high-exposure groups have 13 males with end-stage kidneys). Although THF did not exacerbate development of CPN, it was postulated that it may have exacerbated the development of proliferative lesions within CPN-affected tissue. No data in the peer-reviewed literature are available that support a role of CPN in the induction of THF-induced kidney tumors in male rats.

Doi et al. (2007) concluded that it is possible that α_{2u}-globulin-associated nephropathy may simply contribute to a weak background tumorigenic stimulus provided by age-related chronic progressive nephropathy. However, the study authors stated that there is no direct evidence for the histological alterations, including CPN and ATH, thought to be included with α_{2u}-globulin nephropathy. The overall conclusions of Doi et al. (2007) were that the critical component(s) of the nephropathy most closely associated with the development of tumors cannot clearly be identified. As noted in Section 5.3.6, the mode of action of THF for the male rat kidney tumors has not been determined.

Comments: In an unpublished report submitted in October 19, 2007 to EPA as part of the Public Record, histology slides of kidneys from male and female F344/N rats of the 2-year carcinogenicity and 14-week NTP studies (NTP, 1998) on THF were re-examined (Dammann, 2005; Hard, 2005). The authors of these unpublished reports suggested that the overall incidence of kidney tumors in the male rats was 2/50 (4%), 1/50 (2%), 3/50 (6%), and 5/50 (10%), with all tumors being adenomas (Hard, 2005). Use of the Cochran-Armitage trend test on the data presented in the unpublished report showed no significant concentration-response trend in tumor incidence (Dammann, 2005). The author also concluded that THF does not appear to act via the α_{2u}-globulin mode of action. Instead, the author proposed that advanced CPN may play a role in the development of ATH, and perhaps the kidney tumors from THF exposure.

Additional public comments were submitted to the IRIS Program on July 16, 2009. Included in these comments was a report entitled: "Pathology Working Group Review of Selected Histologic Changes in the Kidneys of Male Rats Assigned to a 2-Year Inhalation Carcinogenicity Study of Tetrahydrofuran (NTP Study No. 05181-03)." The Pathology Working Group (PWG) re-evaluation was conducted during March 3–4, 2009 and included five voting members including Dr. Gordon Hard who had conducted a previous evaluation of the same data. For this discussion, the report will be referred to as PWG (2009). The specified objectives of the new reevaluation were to establish the most appropriate diagnoses of proliferative kidney changes; to provide comment on likely potential pathogenic mechanisms for male rat kidney tumors; and to provide perspective on risk from potential human exposure to THF. In addition to evaluating kidney sections from all the control and high concentration male rat groups, the PWG (2009) examined kidneys that had proliferative lesions in the low and mid exposure male rat groups (5 and 10, respectively). The criteria for proliferative changes were based on Hard et al. (1995). The report by the PWG (2009) stated that the NTP pathologists consolidated all variants of tubular hyperplasia under the diagnostic term "Renal Tubule Hyperplasia." In contrast, the PWG (2009) differentiated between "simple" and "atypical" hyperplasia where, and according to PWG (2009), the first was not recorded because it was regarded as a reactive tubular alteration directly associated with CPN. Atypical tubular hyperplasia (ATH) was recorded by the PWG (2009) because it was considered to represent a potential preneoplastic lesion with strong relevance to carcinogenicity, but severity grades were not assigned.

Both NTP (1998) and the PWG (2009) concluded that renal cell adenomas were increased in the high exposure male rats compared to controls. The PWG (2009) considered both preneoplastic and neoplastic lesions together and reported that when these effects were combined, the incidence values were similar between treated and control rats. In this determination, the PWG (2009) applied different criteria that distinguished between reactive tubular hyperplasia (associated with CPN) and atypical tubule hyperplasia (deemed as preneoplastic). The PWG (2009) concluded that adenomas and ATH were present in kidneys that showed advanced CPN. Furthermore, they suggested that accelerated tubular cell degeneration and regeneration associated with CPN was likely responsible for the development of most proliferative lesions. The PWG (2009) indicated that THF-induced exacerbation of CPN was not considered to be contributory because severity of CPN was similar between treated and control rats. Additionally, the PWG (2009) report stated that there was no evidence of early tumor occurrence or of tumor progression to carcinoma.

The report indicated that the PWG (2009) did not observe histological changes associated with α_{2u}-globulin nephropathy in the chronic NTP (1998) cancer bioassay slides. However, the PWG (2009) report did note that hyaline droplets were detected in the tubular epithelium of high exposure male rats in the subchronic 14-week study, but that similar results were observed in control males. The PWG (2009) also concurred with the results of the BASF (2001a) 4-week inhalation study. Specifically, hyaline droplets were increased in the proximal tubules and hot spots of accelerated cell proliferation were identified in the cortex of male rats exposed to 1,800 ppm THF for 20 days They also noted that immunohistochemistry confirmed that the hyaline droplets contained α_{2u}-globulin following the 4-week inhalation exposure. The PWG (2009) concluded that these slight increases in cell proliferation associated with α_{2u}-globulin may have contributed to the development of adenomas in male rats exposed to the high THF concentration in the chronic cancer bioassay.

The PWG (2009) report concluded that given the absence of data demonstrating statistically significant differences in tumors and preneoplastic lesions, and the assertion that two mechanisms (CPN and α_{2u}-globulin) likely resulted in the proliferative changes observed in the kidney, which have no known counterpart in humans, the formation of renal tubule adenomas in the 2-year carcinogenicity THF study (NTP, 1998) have no relevance to humans.

<u>EPA Response to Comments</u>: EPA agreed with the peer reviewers' comments and continues to conclude that the mode of carcinogenic action for hepatocellular and renal tumors is largely unknown. Additional discussion of the role of CPN and ATH in the development of kidney tumors in male rats observed following exposure to THF has been included in Section 4.7.3.1. EPA disagrees with the characterization in the PWG (2009) report [published as Bruner et al.

(2010)] that renal tubule hyperplasia in the NTP (1998) report is a non-specific term for all variants of tubular hyperplasia and with the approach by Bruner et al. (2010) of combining ATH with neoplastic kidney findings for statistical analyses. There was no difference in the incidence or severity of CPN in male rats of the NTP 2-year carcinogenicity study on THF (both the control and high-dose groups have 13 males with end-stage kidneys). Although THF did not exacerbate development of CPN, it was postulated that it may have exacerbated the development of proliferative lesions within CPN-affected tissue. Specifically, against a background of nephropathy that was uniform across all groups, there were more renal tubular tumors in treated rats than in the controls, and those in the higher exposure group animals were larger in size. Consideration should be given to the robustness and the gender specificity of the renal tumor response. Thus, EPA concluded that the male rat kidney tumors were relevant to humans and that the mode of action for these tumors has not been established.

Comments: A commenter asked why, after concluding in Section 5.3.1 of the external peer review draft Toxicological Review that "quantitative analyses may be useful for providing a sense of the magnitude of potential carcinogenic risk", EPA neglected to revisit this issue after the quantitative cancer assessment was completed. The commenter also questioned why EPA didn't provide greater specificity by discussing what the quantitative results may mean in terms of magnitude of potential carcinogenic risk and whether the results appear sensible for the data. The commenter added that quantitative risk assessment should be reserved for substances where the evidence provides a greater scientific basis for concern, such as human evidence or a confirmed genotoxic mechanism, or at least a clearly defined and established carcinogenic response in multiple test species. The commenter also noted that THF has not been shown to be carcinogenic in humans and has not been confirmed as genotoxic. The commenter added that the male rat renal tumors should not be considered relevant to humans and the hepatic tumors are significantly increased only in the high dose female mice. Based on these considerations, in addition to the absence of any indication of age-dependent susceptibility, the commenter concluded that the application of linear dose-response extrapolation results in an unduly conservative and implausible cancer potency estimate for THF that is comparable in value to two known human carcinogens, namely benzene and vinyl chloride.

EPA Response to Comments: Based on the peer reviewers' concern for the potential overestimation of risk in deriving an IUR for THF using a default linear low-dose extrapolation approach, and the uncertainty associated with the data for THF, EPA did not derive an IUR. See responses to comments under Charge Questions D1 for discussion of the cancer descriptor and quantitative assessment, D2 and D3 regarding modes of action and relevance to humans, and D4 for the discussion of the extrapolation approach.

Comments: A commenter listed three studies (shown below) that were not considered in the draft THF Toxicological Review. Though not considered to likely materially affect the conclusions, the commenter noted that the references should be cited and discussed in the THF Toxicological Review.

- Lehman (2005). Determination of the percutaneous absorption of THF, in vitro, using human cadaver skin model, PRACS Inst., Ltd. (unpublished report provided as #5 supporting document with the comments).
- Loureiro, AP; de Arruda Campos, IP; Gomes, OF; et al. (2005) Structural characterization of an etheno-2'-deoxyguanosine adduct modified by tetrahydrofuran. Chem Res Toxicol 18(2):290–299.
- Hermida, SA; Possari, EP; Souza, DB; et al. (2006) 2'-Deoxyguanosine, 2'-deoxycytidine, and 2'-deoxyadenosine adducts resulting from the reaction of tetrahydrofuran with DNA bases. Chem Res Toxicol 19(7):927–936.

EPA Response to Comments: Conclusions of the studies by Luoriero et al. (2005) and Hermida et al. (2006) have been included with other studies in Section 4.5 (Genotoxicity Studies) and summaries of these studies were included in Appendix C.2. The Lehman (2005) report on a percutaneous absorption model for THF is considered not relevant for this assessment.

APPENDIX B. DOSE-RESPONSE MODELING

The THF data sets considered for dose-response modeling include both quantal and continuous endpoints. EPA's BMDS version 2.0 (U.S. EPA, 2008) was used for model fitting and estimating the benchmark dose (BMD) and its 95% lower bound (i.e., BMDL).

B.1. NONCANCER ENDPOINTS

B.1.1. Methods

Data Sets

For the RfD, the body weight gains for male and female pups of the F1 and F2 generations were considered for dose-response modeling. In order to account for the effect of intralitter correlation on interindividual variability, the individual animal data for the pup body weight gains were analyzed for variance components using SAS PROC NESTED. The standard deviation (SD) for each dose/sex/generation group was estimated using the following equation:

$$\mathrm{SD} = \quad sqrt(\, S^2[1 + (m-1)\rho]\,)$$

where S = SD across all pups in a dose/sex/generation group,
m= weighted average number of pups in a litter in each dose/sex/generation group,
ρ = intralitter correlation.

(see, e.g., Cochran 1977 §9.3; Snedecor and Cochran 1980; Steel and Torrie 1980 §25.7; Winer 1971). The resulting summary statistics, provided in Table B-1, were used as inputs for BMDS continuous models.

For the RfC data sets, see Table 5-3.

Definition of the BMR and corresponding BMD and BMDL.

Rationales for BMRs are provided in Section 5 (Section 5.1.2 for the RfD, Section 5.2.2 for the RfC). For the quantal endpoints, i.e., incidence of cytomegaly, the BMD and BMDL were estimated using a BMR of 10% extra risk. For the continuous endpoints, i.e., pup body weight gain and liver weight, BMD and BMDL were estimated using a BMR of 1SD of the control mean for pup body weight gain, and 10% of the control mean for liver weight. For the selected best-fit models, BMDs and BMDLs were estimated using a BMR of 5% of the control mean for pup body weight gain and 1 SD change in the control mean for liver weight for comparison purposes.

Model selection

For all endpoints, model selection was consistent with EPA's draft *Benchmark Dose Technical Guidance* (U.S. EPA, 2000b). For quantal endpoints, all available dichotomous models in BMDS 2.0 were fit to the incidence data for each dataset. Adequate model fit was judged by the chi-square goodness-of-fit p-value ($p \geq 0.1$). In addition, visual inspection of the fit of the dose-response curve to the data points, especially in the region of the BMR, and examination of the scaled residuals were also used to evaluate model fit. Among all of the models providing adequate fit to the data, the lowest BMDL was selected as the potential POD when the difference between the BMDLs estimated from these models was more than threefold; otherwise, the BMDL from the model with the lowest Akaike's Information Criterion (AIC) was chosen as the candidate POD. The AIC is a measure of the expected deviance of the fit of the specified model from the "true" model that allows for comparison across models allowing for selection of a "best-fit" model for a particular endpoint.

For continuous endpoints, continuous models available in BMDS 2.0 were fit to the data; the Hill model was not considered because it is more useful with more dose groups than were available here. For each model, first the homogeneity of the variances was tested using a likelihood ratio test (BMDS Test 2). If Test 2 was not rejected (χ^2 *p*-value ≥ 0.10), the model was fitted to the data assuming constant variance. If Test 2 was rejected (χ^2 *p*-value < 0.10), the variance was modeled as a power function of the mean, and the variance model was tested using a likelihood ratio test (BMDS Test 3). If Test 3 was not rejected (χ^2 *p*-value ≥ 0.10), the model was fitted to the data using the modeled variance. For fitting models using either constant variance or modeled variance, models were tested for adequacy of fit to the means using a likelihood ratio test (BMDS Test 4, with χ^2 *p*-value < 0.10 indicating inadequate fit). Otherwise the fit of all continuous models were evaluated as described above for the dichotomous models.

Table B-1. F1 and F2 Pup body weight gain changes for RfD derivation from the two-generation reproductive toxicity study in Wistar rats exposed to THF in drinking water

Generation, sex	Parameter	Concentration (ppm)			
		0	1,000	3,000	9,000
F0 Generation/F1 Pups					
F0 Females	TWA THF intake[a]	0	128	362	1014
F1 Male pups	Pup body weight gain (g) PND 7−14	17.8 ± 2.4[c] (101)	17.4 ± 2.8 (77)	17.1 ± 2.8 (90)	15.7 ± 3.4 (94)
F1 Female pups	Pup body weight gain (g) PND 7−14	17.4 ± 2.9 (90)	17.3 ± 2.9 (88)	16.9 ± 2.8 (83)	15.6 ± 3.0 (89)
F1 Generation/F2 Pups					
F1 Females	TWA THF intake[a]	0	125	371	937
F2 Male pups	Pup body weight gain (g) PND 7−14	17.4 ± 3.1 (95)	17.7 ± 3.4 (76)	16.9 ± 3.9 (91)	15.6 ± 3.1 (79)
F2 Female pups	Pup body weight gain (g) PND 7−14	17.1 ± 2.9 (93)	17.3 ± 3.4 (74)	16.2 ± 4.2 (88)	15.3 ± 3.3 (85)

[a] Time-weighted average (TWA) during gestation through Day 14 of lactation (mg/kg-day); see Table 4-6 for additional details.
[b] Mean ± SD and (number of pups), where the mean is the mean across all pups within a study group and the SD reflects interindividual variability and intralitter correlation.

Sources: Hellwig et al. (2002); BASF (1996).

B.1.2. Modeling Results for Noncancer Effects Resulting from Oral Exposure

Table B-2. Dose-response modeling results for pup body weight gain in the Wistar rat two-generation reproductive toxicity study of orally administered THF

Endpoint and model	AIC[a]	*p*-Value	BMD_{1SD} mg/kg-day	$BMDL_{1SD}$[b] mg/kg-day	BMD_5 mg/kg-day	$BMDL_5$[b] mg/kg-day
Pup body weight gain F1 males, PND 7–14						
Linear, Power (power ≥1)[c]	**1113.3**	**0.67**	**1257**	**928**	**448**	**338**
Polynomial (2-degree)[c]	1115.3	0.37	1255	928		
Polynomial (3-degree)[c]	1115.3	0.38	1233	928		
Pup body weight gain F1 females, PND 7–14						
Linear[d]	**1089.4**	**0.92**	**1559**	**1152**	**478**	**359**
Power (power ≥1)[d]	1091.2	0.92	1454	1034		
Polynomial (2-degree)[d]	1091.2	0.88	1398	1125		
Pup body weight gain F2 males, PND 7–14						
Linear[d]	**1175.1**	**0.54**	**1589**	**1131**	**416**	**302**
Power (power ≥1)[d]	1176.6	0.38	1376	961		
Polynomial (2-degree)[d]	1176.7	0.35	1319	1064		
Pup body weight gain F2 females, PND 7–14						
Linear[d]	**1194.3**	**0.53**	**1661**	**1174**	**410**	**296**
Polynomial (2-degree)[d]	1194.3	0.53	1661	1138		
Power (power ≥1)[d]	1194.3	0.53	1661	1156		

[a]AIC = Akaike Information Criterion (see B.1.1).
[b]BMDL = 95% lower bound of the BMD. Subscript denotes the specified benchmark response (BMR) level, a 1 SD (standard deviation) or 5% decrease from the control mean. Results are shown rounded to the nearest mg/kg/day. The results for the best-fitting models for pup body weight gain are shown in bold font.
[c] Variance modeled.
[d] Constant variance.
[e] Variances could not be modeled adequately (BMDS Test 3 p = 0.068 with variance constant and p = 0.032 with variance modeled). The constant variance model was used. The means fit adequately in either case (BMDS Test 4 p-value is in the table) and the BMDs and BMDLs were similar across both fits.
[f] Variances could not be modeled adequately (BMDS Test 3 p = 0.0038 with variance constant and p = 0.0087 with variance modeled). The means fit adequately and AIC was lower for the constant variance models; the constant variance model was used. The BMDs and BMDLs were similar across both fits.

Sources: Based on data from Hellwig et al. (2002); BASF (1996).

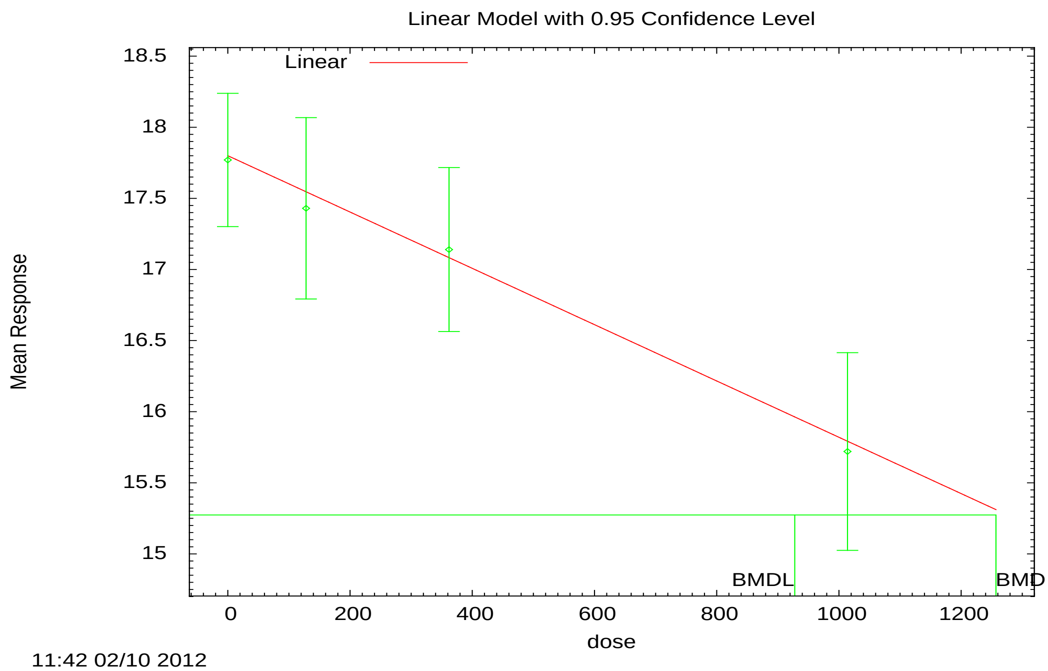

Figure B-1. Pup body weight gain, F1 male rats. BMR=1 SD.

Source: Based on data from Hellwig et al. (2002); BASF (1996).

```
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     Polynomial Model. (Version: 2.16;  Date: 05/26/2010)
     Input Data File: C:\Documents and Settings\jfox\My Documents\_CURRENTWORK\Work
   Assignments\WA 67\THF data\BMDS22.THF.F1.1SD\lin_THF_BASF_F1M_wtgn_PND7-14_Lin-ModelVariance-
   BMR1Std.(d)
     Gnuplot Plotting File: C:\Documents and Settings\jfox\My Documents\_CURRENTWORK\Work
   Assignments\WA 67\THF data\BMDS22.THF.F1.1SD\lin_THF_BASF_F1M_wtgn_PND7-14_Lin-ModelVariance-
   BMR1Std.plt
                                          Fri Feb 10 11:42:00 2012
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 BMDS Model Run
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   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = Mean
   Independent variable = Dose
   Signs of the polynomial coefficients are not restricted
   The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                         lalpha =       2.09468
                            rho =             0
                         beta_0 =       17.7636
                         beta_1 =   -0.00199102
```

Asymptotic Correlation Matrix of Parameter Estimates

	lalpha	rho	beta_0	beta_1
lalpha	1	-1	-0.015	0.024
rho	-1	1	0.015	-0.024
beta_0	-0.015	0.015	1	-0.64
beta_1	0.024	-0.024	-0.64	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
lalpha	16.6383	4.49961	7.81922	25.4574
rho	-5.14883	1.58825	-8.26173	-2.03592
beta_0	17.7635	0.187513	17.3959	18.131
beta_1	-0.00198088	0.000405902	-0.00277643	-0.00118532

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	101	17.8	17.8	2.37	2.49	0.0264
128	77	17.4	17.5	2.81	2.58	-0.271
362	90	17.1	17	2.75	2.77	0.321
1014	94	15.7	15.8	3.39	3.39	-0.0997

Model Descriptions for likelihoods calculated

```
Model A1:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2

Model A2:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma(i)^2

Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
    Model A3 uses any fixed variance parameters that
    were specified by the user

Model  R:         Yi = Mu + e(i)
           Var{e(i)} = Sigma^2
```

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-558.125114	5	1126.250229
A2	-551.789435	8	1119.578869
A3	-552.253701	6	1116.507402
fitted	-552.648862	4	1113.297724
R	-571.895300	2	1147.790600

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)

```
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              40.2117          6          <.0001
   Test 2              12.6714          3        0.005404
   Test 3             0.928533          2          0.6286
   Test 4             0.790322          2          0.6736

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data



             Benchmark Dose Computation

Specified effect =             1

Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =       1256.91


            BMDL =       927.705


Specified effect =          0.05

Risk Type        =     Relative risk

Confidence level =          0.95

             BMD =       448.374


            BMDL =       338.318
```

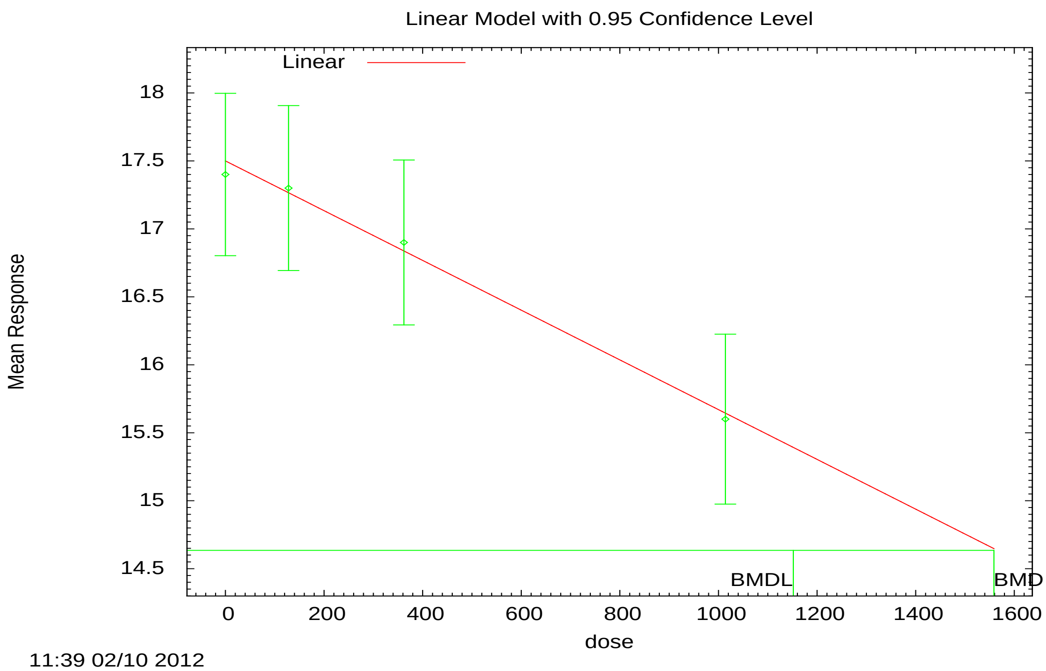

Figure B-2. Pup body weight gain, F1 female rats. BMR=1 SD.

Source: Based on data from Hellwig et al. (2002); BASF (1996).

```
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     Polynomial Model. (Version: 2.16;  Date: 05/26/2010)
     Input Data File: C:\Documents and Settings\jfox\My Documents\_CURRENTWORK\Work
   Assignments\WA 67\THF data\BMDS22.THF.F1.1SD\lin_THF_BASF_F1F_wtgn_PND7-14_Lin-
   ConstantVariance-BMR1Std.(d)
     Gnuplot Plotting File: C:\Documents and Settings\jfox\My Documents\_CURRENTWORK\Work
   Assignments\WA 67\THF data\BMDS22.THF.F1.1SD\lin_THF_BASF_F1F_wtgn_PND7-14_Lin-
   ConstantVariance-BMR1Std.plt
                                          Fri Feb 10 11:39:35 2012
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 BMDS Model Run
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   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = Mean
   Independent variable = Dose
   rho is set to 0
   Signs of the polynomial coefficients are not restricted
   A constant variance model is fit

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                          alpha =      8.21839
                            rho =            0   Specified
                         beta_0 =      17.4881
                         beta_1 =  -0.00183013


          Asymptotic Correlation Matrix of Parameter Estimates
```

(*** The model parameter(s) -rho
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	alpha	beta_0	beta_1
alpha	1	-1.2e-010	6.3e-009
beta_0	-1.2e-010	1	-0.69
beta_1	6.3e-009	-0.69	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	8.12857	0.614462	6.92425	9.3329
beta_0	17.4861	0.210578	17.0734	17.8988
beta_1	-0.0018292	0.000386625	-0.00258697	-0.00107143

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	90	17.4	17.5	2.85	2.85	-0.287
128	88	17.3	17.3	2.86	2.85	0.158
362	83	16.9	16.8	2.78	2.85	0.243
1014	89	15.6	15.6	2.97	2.85	-0.104

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-541.603958	5	1093.207915
A2	-541.409197	8	1098.818394
A3	-541.603958	5	1093.207915
fitted	-541.692415	3	1089.384830
R	-552.541184	2	1109.082368

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)

Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	22.264	6	0.001084
Test 2	0.389521	3	0.9424
Test 3	0.389521	3	0.9424
Test 4	0.176914	2	0.9153

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels It seems appropriate to model the data

The p-value for Test 2 is greater than .1. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation

Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 1558.64

BMDL = 1151.82

Specified effect = 0.05

Risk Type = Relative risk

Confidence level = 0.95

BMD = 477.972

BMDL = 359.199

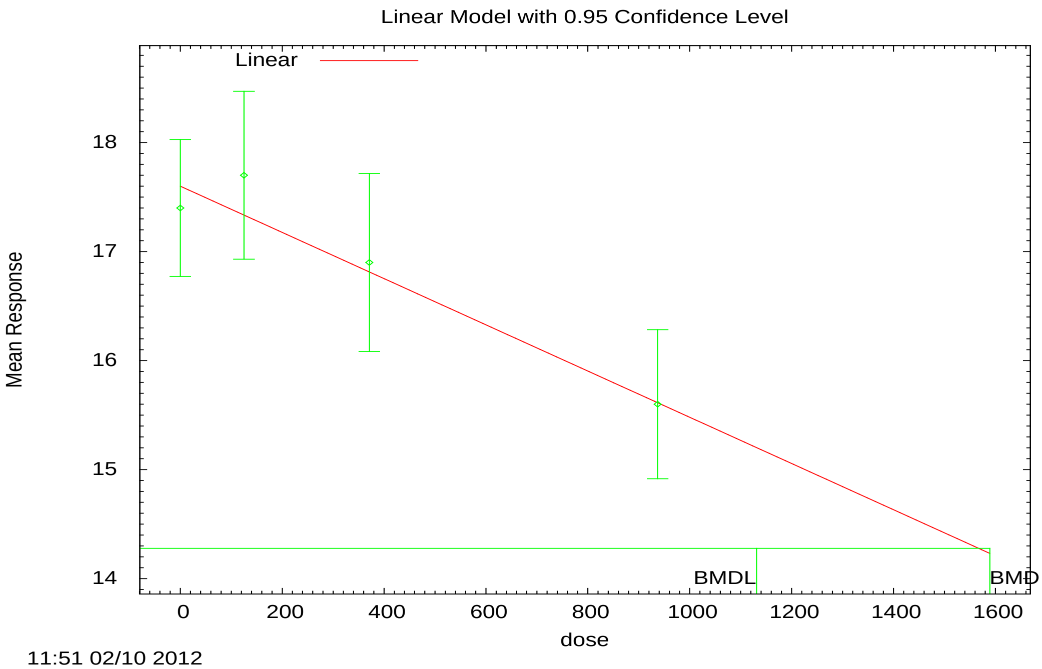

Figure B-3. Pup body weight gain, F2 male rats. BMR=1 SD.

Source: Based on data from Hellwig et al. (2002); BASF (1996).

```
 ====================================================================
      Polynomial Model. (Version: 2.16;  Date: 05/26/2010)
      Input Data File: C:\Documents and Settings\jfox\My Documents\_CURRENTWORK\Work
    Assignments\WA 67\THF data\BMDS22.THF.F2.1SD\lin_THF_BASF_F2M_wtgn_PND7-14_Lin-
    ConstantVariance-BMR1Std.(d)
      Gnuplot Plotting File: C:\Documents and Settings\jfox\My Documents\_CURRENTWORK\Work
    Assignments\WA 67\THF data\BMDS22.THF.F2.1SD\lin_THF_BASF_F2M_wtgn_PND7-14_Lin-
    ConstantVariance-BMR1Std.plt
 ====================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = Mean
   Independent variable = Dose
   rho is set to 0
   Signs of the polynomial coefficients are not restricted
   A constant variance model is fit

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                          alpha =      11.4329
                            rho =            0   Specified
                         beta_0 =      17.6735
                         beta_1 =  -0.00215907
```

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -rho
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	alpha	beta_0	beta_1
alpha	1	-3.8e-010	1.6e-011
beta_0	-3.8e-010	1	-0.7
beta_1	1.6e-011	-0.7	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	11.3396	0.86843	9.63749	13.0417
beta_0	17.6453	0.254252	17.1469	18.1436
beta_1	-0.00211908	0.000515127	-0.00312871	-0.00110945

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	95	17.4	17.6	3.08	3.37	-0.71
125	76	17.7	17.4	3.37	3.37	0.827
371	91	16.9	16.9	3.92	3.37	0.116
937	79	15.6	15.7	3.05	3.37	-0.158

Model Descriptions for likelihoods calculated

```
Model A1:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2

Model A2:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma(i)^2

Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2
    Model A3 uses any fixed variance parameters that
    were specified by the user

Model  R:         Yi = Mu + e(i)
           Var{e(i)} = Sigma^2
```

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-583.910529	5	1177.821058
A2	-580.159687	8	1176.319374
A3	-583.910529	5	1177.821058
fitted	-584.525080	3	1175.050160
R	-592.783141	2	1189.566283

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)

Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	25.2469	6	0.0003073
Test 2	7.50168	3	0.05752
Test 3	7.50168	3	0.05752
Test 4	1.2291	2	0.5409

The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1. Consider running a
non-homogeneous variance model

The p-value for Test 3 is less than .1. You may want to consider a
different variance model

The p-value for Test 4 is greater than .1. The model chosen seems
to adequately describe the data

Benchmark Dose Computation

Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 1589.1

BMDL = 1131.2

Specified effect = 0.05

Risk Type = Relative risk

Confidence level = 0.95

BMD = 416.342

BMDL = 302.013

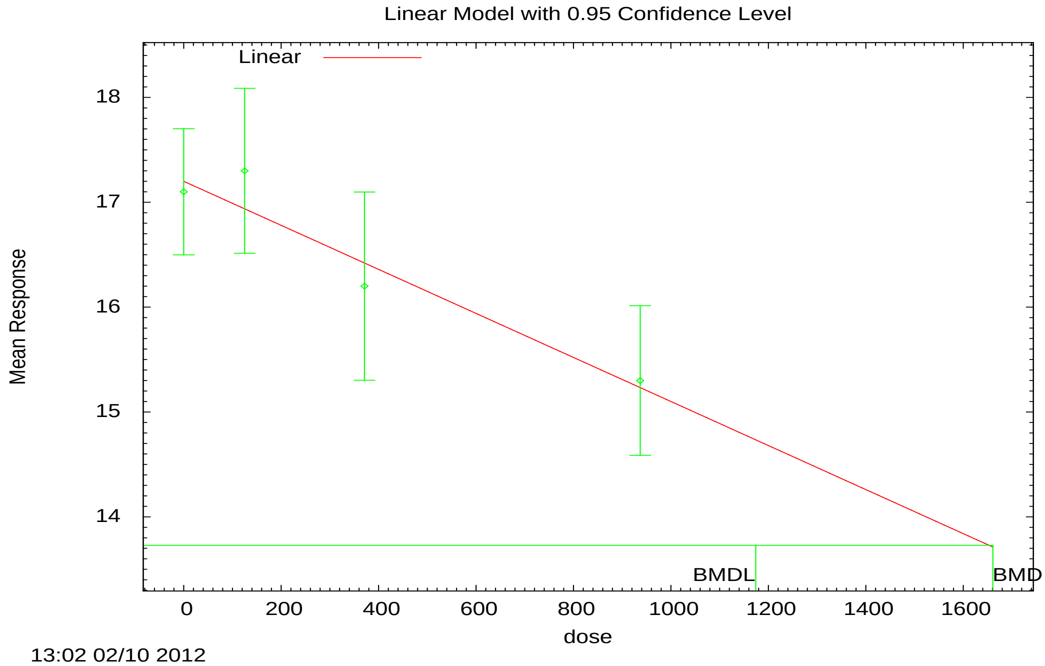

Figure B-4. Pup body weight gain, F2 female rats. BMR=1 SD.

Source: Based on data from Hellwig et al. (2002); BASF (1996).

```
====================================================================
         Polynomial Model. (Version: 2.16;  Date: 05/26/2010)
         Input Data File: C:\Documents and Settings\jfox\My Documents\_CURRENTWORK\Work
Assignments\WA 67\THF data\BMDS22.THF.F2.1SD\lin_THF_BASF_F2F_wtgn_PND7-14_Lin-ConstantVariance-
BMR1Std.(d)
         Gnuplot Plotting File: C:\Documents and Settings\jfox\My Documents\_CURRENTWORK\Work
Assignments\WA 67\THF data\BMDS22.THF.F2.1SD\lin_THF_BASF_F2F_wtgn_PND7-14_Lin-ConstantVariance-
BMR1Std.plt
====================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = Mean
   Independent variable = Dose
   rho is set to 0
   Signs of the polynomial coefficients are not restricted
   A constant variance model is fit

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                          alpha =      12.2212
                            rho =            0   Specified
                         beta_0 =      17.2359
                         beta_1 =  -0.00212387
```

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -rho
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	alpha	beta_0	beta_1
alpha	1	3.3e-009	-2e-009
beta_0	3.3e-009	1	-0.7
beta_1	-2e-009	-0.7	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	12.1224	0.929743	10.3001	13.9446
beta_0	17.21	0.265533	16.6896	17.7304
beta_1	-0.00209639	0.000522243	-0.00311997	-0.00107281

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	93	17.1	17.2	2.92	3.48	-0.305
125	74	17.3	16.9	3.4	3.48	0.87
371	88	16.2	16.4	4.24	3.48	-0.626
937	85	15.3	15.2	3.31	3.48	0.144

Model Descriptions for likelihoods calculated

```
Model A1:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2

Model A2:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma(i)^2

Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2
    Model A3 uses any fixed variance parameters that
    were specified by the user

Model  R:         Yi = Mu + e(i)
           Var{e(i)} = Sigma^2
```

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-593.526954	5	1197.053908
A2	-586.818614	8	1189.637228
A3	-593.526954	5	1197.053908
fitted	-594.158936	3	1194.317873
R	-602.030764	2	1208.061528

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)

```
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              30.4243          6          <.0001
   Test 2              13.4167          3        0.003817
   Test 3              13.4167          3        0.003817
   Test 4              1.26396          2          0.5315

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  Consider running a
non-homogeneous variance model

The p-value for Test 3 is less than .1.  You may want to consider a
different variance model

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


             Benchmark Dose Computation

Specified effect =             1

Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =       1660.81


            BMDL =       1174.05



Specified effect =          0.05

Risk Type        =     Relative risk

Confidence level =          0.95

             BMD =       410.467


            BMDL =       296.025
```

B.1.3. Modeling Results for Noncancer Effects Resulting from Inhalation Exposure

Table B-3. Dose-response modeling[a] results for noncancer effects resulting from subchronic inhalation exposure to THF

Male mice: liver weight						
Model	**AIC**	***p*-value**	**BMC_{1SD}**	**$BMCL_{1SD}$**	**BMC_{10}[b]**	**$BMCL_{10}$[b]**
Power (unrestricted)	**-190.1**	**0.81**	**374**	**80**	**783**	**246**
Hill	-189.0	0.55	607	275	1030	502
Linear (and higher order polynomials)	-189.9	0.53	912	710	1390	1110
Male mice: centrilobular cytomegaly						
Model	**AIC**	***p*-value**			**BMC_{10}**	**$BMCL_{10}$**
Gamma, Weibull (power ≥1)	22.72	1.0			948	266
Log-logistic (slope ≥1)	22.72	1.0			948	322
Logistic	23.04	0.66			1138	645
Multistage, degree 2 (coefficients ≥0)	**20.86**	**0.93**			**805**	**256**
Probit	22.89	0.75			1061	602
Log-probit	22.72	1.0			948	358

[a]Concentrations used in the modeling were the HECs in mg/m^3. The results for the best-fitting models for absolute liver weight and cytomegaly are shown in bold font.
[b]For the liver weight endpoints, $BMC_{10}/BMCL_{10}$ refers to a 10% relative increase from the control value. For liver pathology, $BMC_{10}/BMCL_{10}$ refers to 10% extra risk in incidence of centrilobular cytomegaly.

Source: Based on data from NTP (1998).

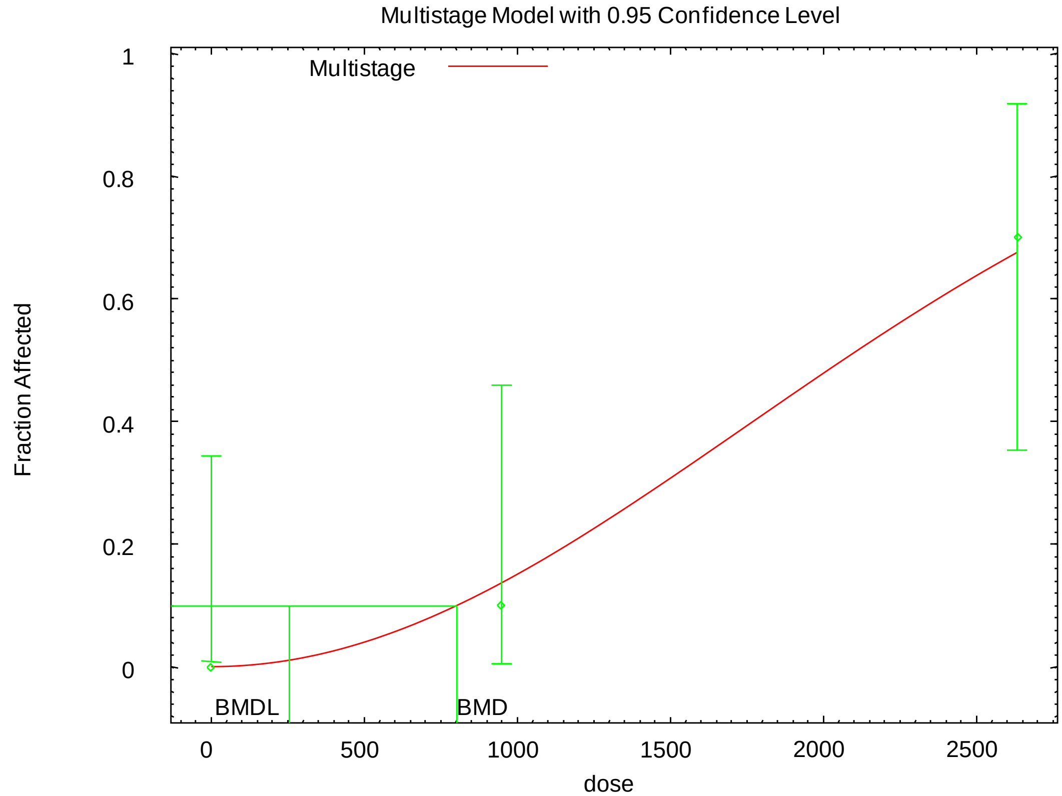

Figure B-5. Liver centrilobular cytomegaly, male mice.

Source: Based on data from NTP (1998).

```
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
Polynomial Model
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
-beta1*dose^1-beta2*dose^2)]

   The parameter betas are restricted to be positive

   Dependent variable = incidence
   Independent variable = dose

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2

 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008

                  Default Initial Parameter Values
                     Background =            0
```

```
                        Beta(1) =            0
                        Beta(2) = 1.76579e-007

           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Background    -Beta(1)
                 have been estimated at a boundary point, or have been specified by the user, and
do not appear in the correlation matrix )

                Beta(2)
   Beta(2)            1

                          Parameter Estimates
       Variable           Estimate            Std. Err.
     Background                  0                 NA
        Beta(1)                  0                 NA
        Beta(2)       1.62776e-007        7.75991e-008

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.

                        Analysis of Deviance Table
       Model      Log(likelihood)  Deviance  Test DF     P-value
     Full model        -9.35947
   Fitted model        -9.43218      0.145417      2          0.9299
  Reduced model        -17.3975        16.076      2        0.000323
           AIC:         20.8644

                     Goodness  of  Fit
     Dose     Est._Prob.    Expected    Observed     Size     Chi^2 Res.
  ----------------------------------------------------------------------
    0.0000     0.0000         0.000         0          10       0.000
  948.0000     0.1361         1.361         1          10      -0.307
 2634.0000     0.6768         6.768         7          10       0.106
 Chi-square =       0.14     DF = 2        P-value = 0.9345

   Benchmark Dose Computation
Confidence level =          0.95
Specified effect =           0.1
Risk Type        =      Extra risk
             BMD =        804.532
            BMDL =          255.8
```

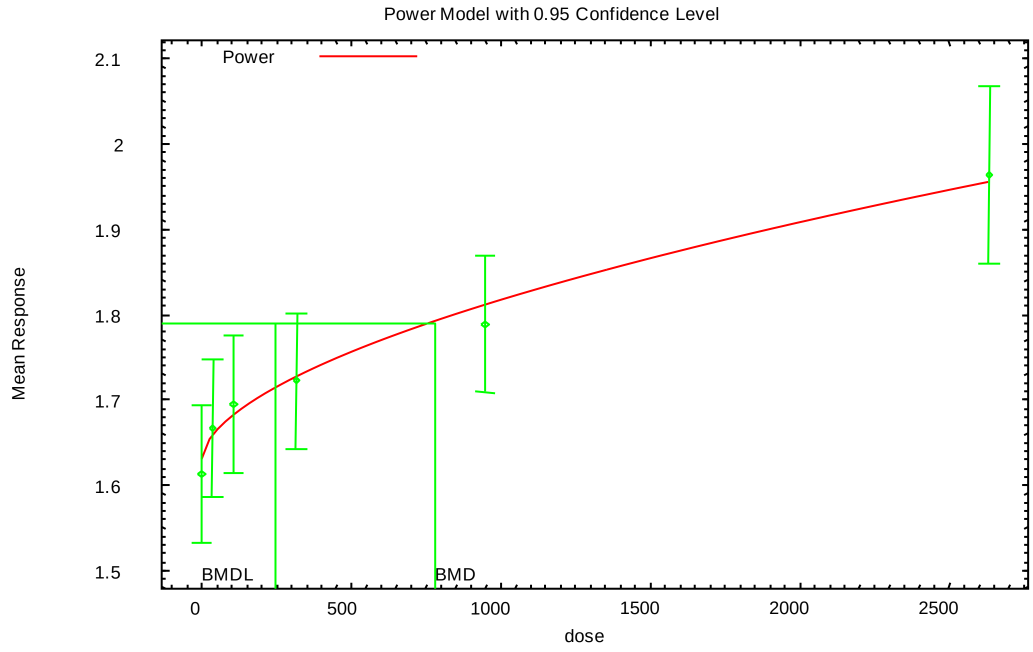

Figure B-6. Absolute liver weight, male mice.

Source: Based on data from NTP (1998)

```
.
====================================================================
          Power Model.
 ====================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = control + slope * dose^power

   Dependent variable = MEAN
   Independent variable = mg_cum_hec
   rho is set to 0
   The power is not restricted
   A constant variance model is fit

   Total number of dose groups = 6
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                  Default Initial Parameter Values
                          alpha =    0.0125082
                            rho =            0   Specified
                        control =        1.613
                          slope =    0.0133873
                          power =     0.414725
```

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -rho
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	alpha	control	slope	power
alpha	1	-1.8e-009	-4.8e-009	6.2e-009
control	-1.8e-009	1	-0.82	0.79
slope	-4.8e-009	-0.82	1	-1
power	6.2e-009	0.79	-1	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	0.0113839	0.00213239	0.00720445	0.0155633
control	1.62729	0.032356	1.56387	1.69071
slope	0.00362732	0.00585053	-0.0078395	0.0150941
power	0.5708	0.202297	0.174305	0.967294

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	10	1.61	1.63	0.117	0.107	-0.424
35	10	1.67	1.65	0.0696	0.107	0.359
105	10	1.7	1.68	0.117	0.107	0.475
316	10	1.72	1.72	0.098	0.107	-0.0655
948	10	1.79	1.81	0.111	0.107	-0.585
2634	7	1.96	1.95	0.159	0.107	0.287

Model Descriptions for likelihoods calculated

```
Model A1:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2

Model A2:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma(i)^2

Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2
    Model A3 uses any fixed variance parameters that
    were specified by the user

Model  R:         Yi = Mu + e(i)
           Var{e(i)} = Sigma^2
```

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	99.538919	7	-185.077839
A2	102.357731	12	-180.715462
A3	99.538919	7	-185.077839
fitted	99.053425	4	-190.106851
R	80.470340	2	-156.940680

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)

Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	43.7748	10	<.0001
Test 2	5.63762	5	0.3431
Test 3	5.63762	5	0.3431
Test 4	0.970988	3	0.8083

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels It seems appropriate to model the data

The p-value for Test 2 is greater than .1. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Relative risk

Confidence level = 0.95

BMD = 783.381

BMDL = 246.114

Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 373.946

BMDL = 79.9732

B.2. DERIVATION OF AN INHALATION CANCER RISK ESTIMATE USING A LINEAR LOW-DOSE EXTRAPOLATION APPROACH FOR THF

B.2.1. Rationale and Methods for Deriving a Cancer Risk Estimate

As indicated in Section 5.3.1., the U.S. EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) state: "When there is suggestive evidence, the Agency generally would not attempt a dose-response assessment, as the nature of the data generally would not support one; however, when the evidence includes a well-conducted study, quantitative analyses may be useful for some purposes, for example, providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities. In each case, the rationale for the quantitative analysis is explained, considering the uncertainty in the data and the suggestive nature of the weight of evidence. These analyses generally would not be considered Agency consensus estimates."

In this case, the NTP (1998) cancer bioassay for THF is a well-conducted study showing hepatocellular adenoma or carcinomas in female mice that were increased in a dose-related manner, starting at an approximate 15% increase over controls at the lowest exposure. The adjusted rate of renal adenoma or carcinoma in male rats was also increased in a dose-related manner, starting at an approximate 9% increase over controls at the lowest exposure. The data from this study are amenable to modeling; EPA would generally derive a cancer risk estimate from such data.

However, a majority of the external peer review panel members (see Appendix A: Summary of External Peer Review and Public Comments and Disposition) stated that derivation of an inhalation unit risk (IUR) for THF would result in an overestimation of cancer risk if a linear low-dose extrapolation approach was utilized. Although the reviewers agreed with EPA's conclusion that based on the available data the modes of action for both liver and kidney tumors induced by THF are not well understood, they suggested that THF is a weak, nongenotoxic carcinogen that would have a threshold. The U.S. EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) recommend that the method used to characterize and quantify cancer risk for a chemical is determined by what is known about the mode of action of the carcinogen and the shape of the cancer dose-response curve. The linear approach is recommended if the mode of action of carcinogenicity is not understood (U.S. EPA, 2005a). In the case of THF, very little data are available to inform the mode of action and no data are available to indicate the shape of the dose-response curve at low doses. If data were available to better inform the mode of action, and the data were indicative of a threshold response, then a reference value could be derived based on a precursor endpoint (i.e., key event in the mode of action) and considered for the RfC for THF. For THF, there were no reported noncancer effects that could serve as a precursor endpoint upon which to base a nonlinear analysis. Thus, the nonlinear analysis recommended by the peer reviewers cannot be readily implemented.

Based on the peer reviewers' concern for the potential overestimation of risk in deriving an IUR for THF using a linear low-dose extrapolation approach combined with the uncertainty associated with the carcinogenic potential for THF, EPA did not derive an IUR. However, because there may be some circumstances, as indicated in the cancer guidelines, for which a cancer risk estimate for THF would be useful, EPA has presented what the inhalation cancer risk estimate would be if it were derived using a linear low-dose approach.

Table B-3 summarizes both the overall and survival-adjusted incidences of mouse hepatocellular and rat renal neoplasms from the NTP (1998) study. Adenoma and carcinoma incidences within each site were combined by counting animals with either of these responses. This practice was performed under the assumption that adenomas and carcinomas originating from the same cell type represent stages along a continuum of carcinogenic effects resulting from the same mechanism, as recommended in EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a).

Table B-3. Incidences of neoplastic lesions of the livers of female $B6C3F_1$ mice and kidneys of male F344/N rats exposed via inhalation to THF 6 hours/day, 5 days/week for 105 weeks (NTP, 1998)

Lesion	Concentration (ppm)			
	0	**200**	**600**	**1,800**
Female $B6C3F_1$ mice				
Hepatocellular adenoma or carcinoma				
Overall incidence[a]	17/50	24/50	26/50	41/48
Adjusted rate[b]	46.3%	61.3%	69.1%	93.0%
Adjusted incidence[c]	17/37	24/39	26/38	41/44
Trend test *p*-values[d]	p <0.001			
Male F344/N rats				
Renal adenoma or carcinoma				
Overall incidence[a]	1/50	1/50	4/50	5/50
Adjusted rate[b]	8.3%	16.7%	18.8%	38.3%
Adjusted incidence[c]	1/12	1/6	4/21	5/13
Trend test *p*-values[d]	p <0.037			

[a]Number of animals with tumors per number of animals examined.
[b]Kaplan-Meier estimated tumor incidence at the end of the study, incorporating an adjustment for intercurrent mortality.
[c]Adjusted denominator estimated by dividing numerator (tumors) by the adjusted rate expressed as a proportion (e.g., 0.083 rather than 8.3%).
[d]Trend tests: logistic regression.

Source: NTP (1998).

THF is water soluble, and toxicokinetic information suggests that it is systemically absorbed and widely distributed following inhalation exposure in both humans and animals (Droz et al., 1999; Ong et al., 1991; Kageyama, 1988; Elovaara et al., 1984; Kawata and Ito, 1984; Wagner, 1974). Accordingly, the liver and kidney tumors observed following inhalation exposure to THF are considered extrarespiratory effects of a category 3 gas as defined by EPA's RfC Methodology: *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b). Experimental exposure concentrations were converted to mg/m^3 (0, 590, 1,770, and 5,310 mg/m^3), and adjusted to a continuous exposure basis ($mg/m^3 \times 6$ hours/24 hours $\times$ 5 days/7 days = $mg/m^3 \times 0.1786$: 0, 105, 316, and 948 mg/m^3). For category 3 gas equations, HECs are calculated by multiplying the duration-adjusted exposure concentration by the regional gas dose ratio (RGDR) for the extrarespiratory region. The RGDR for extrarespiratory effects is calculated by finding the ratio of the animal-to-human blood:gas (air) partition coefficients. In cases where there are either no data available or where the animal partition coefficient is larger than the human coefficient, a default value of 1 is used for the RGDR. For THF, a human blood:gas partition coefficient was available from Ong et al. (1991); however, no value was available for animals. Therefore, the default of 1 was applied in estimating the HECs for extrarespiratory effects.

Because there are no biologically based dose-response models suitable for the tumor data identified above, the data sets for incidence of hepatocellular adenoma or carcinoma observed in female B6C3F$_1$ mice and for incidence of renal tubule adenoma or carcinoma in male F344/N rats, both adjusted for intercurrent mortality as estimated by NTP (1998) (see Table B-3), were modeled using the cancer-multistage model in the BMDS version 2.0 (U.S. EPA, 2008). A 10% BMR was used with each tumor type (U.S. EPA, 2005a). The multistage model was run for all polynomial degrees up to n-1 (where n is the number of dose groups including control).

B.2.2. Modeling Results for Cancer Effects Resulting from Inhalation Exposure

Modeling results are shown in Table B-4 (modeling details provided in Section B.2.3).

Table B-4. Cancer multistage modeling results for THF

Endpoint	*p*-Value	$BMC_{10/HEC}$ [a]	$BMCL_{10/HEC}$ [a]
Hepatocellular adenoma or carcinoma (female mice)	0.47	52	35
Renal tubule adenoma or carcinoma (male rats)	0.59	260	127

[a]Concentrations used in the modeling were the HECs reported in mg/m^3 and assuming the ratio of animal to human air:blood partition coefficients is 1.

Source: Modeling based on data from NTP (1998).

In both cases, the one-stage multistage model provided adequate data fits with goodness-of-fit *p*-values higher than 0.05; consequently, these results were used since there was no compelling biological reason to use another empirical model. For the hepatocellular adenoma or carcinoma data set, the $BMC_{10/HEC}$ and $BMCL_{10/HEC}$ are 52 and 35 mg/m^3, respectively. For the renal tubule adenoma or carcinoma data in male F344/N rats, the model gives the $BMC_{10/HEC}$ of 260 mg/m^3 and corresponding $BMCL_{10/HEC}$ of 127 mg/m^3.

Development of an inhalation cancer risk estimate for THF would be based on the data for female mouse liver tumors since these data provided the strongest carcinogenic response to inhalation exposure in animals. Thus, the $BMCL_{10/HEC}$ of 35 mg/m^3 would serve as the POD. As noted above, in the absence of information to establish the mode of carcinogenic action and to inform the shape of the cancer dose-response curve, and under the U.S. EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), EPA concluded that the data are insufficient to provide significant biological support for either a linear or a nonlinear extrapolation approach; thus, a default linear approach would be applied.

An inhalation cancer risk estimate is derived from the $BMCL_{10/HEC}$ (the lower bound on the exposure associated with a 10% extra cancer risk) by dividing the risk (as a fraction) by the $BMCL_{10/HEC}$ and represents an upper bound estimate on human extra cancer risk from continuous lifetime inhalation exposure to THF. The $BMCL_{10/HEC}$ for extra risk of hepatocellular adenomas or carcinomas in female $B6C3F_1$ mice exposed to THF results in a risk estimate of $0.1/(35\ mg/m^3) = 0.0029\ (mg/m^3)^{-1}$ or 3×10^{-6} per $\mu g/m^3$ (rounded to one significant figure). This value was derived by linear extrapolation to the origin from the POD of 35 mg/m^3 and represents an upper bound estimate. This inhalation cancer risk value should not be used with exposures >35 mg/m^3, because above this level, the modeled dose-response relationship better characterizes what is known about the carcinogenicity of THF rather than the risk estimate. The slope of the linear extrapolation from the BMC_{10}, associated with 10% extra cancer risk, is calculated as $0.1/(52\ mg/m^3) = 0.0019\ (mg/m^3)^{-1}$ or 2×10^{-6} per $\mu g/m^3$. Risk assessors should use caution when considering the use of the inhalation cancer risk estimate, due to the uncertainty associated with the potential overestimation of risk related to the linear low-dose extrapolation approach employed in its derivation and the suggestive nature of the tumorigenic response.

B.2.3. Details of Modeling Results for Cancer Effects

Table B-5. Summary of model selection and modeling results for best-fitting multistage models for cancer effects resulting from chronic inhalation exposure to THF reported by NTP (1998)

Data Set	Degree of Model	df	Goodness-of-fit *p*-value	LL[a]	χ^{2b}	BMC_{10} (mg/m^3)	$BMCL_{10}$ (mg/m^3)	Model selection rationale[c]
Female mouse hepatocellular tumors	**1** 2 3	**1** 2 2	**0.95** 0.75 0.76	–86.4707 –86.4345 –86.4118	NR 0.07 0.05	**51.7** 61.4 61.2	**35.2** 35.4 35.5	**Most parsimonious fit**
Male rat kidney tumors	**1** 2 3	**1** 2 2	**0.50** 0.38 0.43	–25.0786 –25.0783 –25.0775	NR <0.1 <0.1	**260** 268 273	**127** 127 127	**Most parsimonious fit**

The results for the best-fitting degree of multistage model for hepatocellular and kidney tumors are shown in bold font. See the following BMDS outputs for further details.

[a]LL=Log-likelihood.
[b]$\chi^2 = 2 \times |(LL_i - LL_j)|$, where i and j are consecutive numbers of stages. The test was evaluated for 1 degree of freedom (df). χ^2 for 1 df at $\alpha = 0.05$ is 3.84. NR=not reported.
[c]Adequate fit: goodness-of-fit *p* >0.05, scaled residuals <2.0, good fit near BMR, lack of extreme curvature not reflected in the observed data. Parsimonious=No statistical improvement in fit with higher degree models.

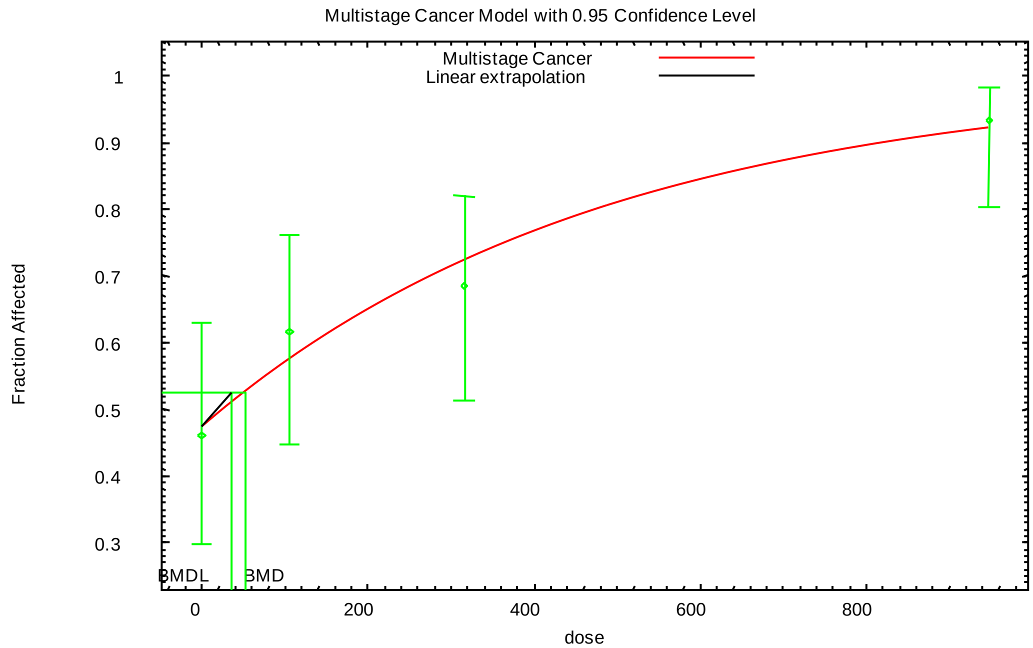

Figure B-7. Hepatocellular adenomas or carcinomas, female mice.

Source: Based on data from NTP (1998).

```
 ====================================================================
         Multistage Cancer Model
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 BMDS_Model_Run
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Effect
   Independent variable = dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =     0.461466
                        Beta(1) =   0.00214268
```

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.56
Beta(1)	-0.56	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.473223	*	*	*
Beta(1)	0.00203941	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-86.1605	4			
Fitted model	-86.4707	2	0.6204	2	0.7333
Reduced model	-98.6187	1	24.9164	3	<.0001

AIC: 176.941

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.4732	17.509	17.000	37	-0.168
105.0000	0.5748	22.416	24.000	39	0.513
316.0000	0.7235	27.492	26.000	38	-0.541
948.0000	0.9238	40.647	41.000	44	0.201

Chi^2 = 0.62 d.f. = 2 P-value = 0.7319

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 51.6621

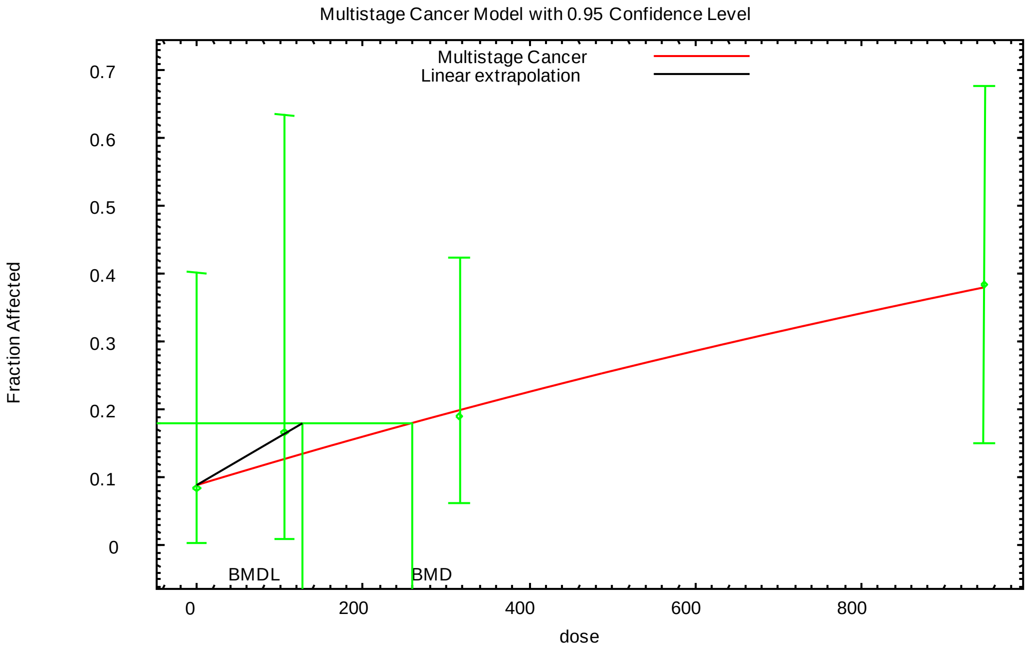
BMDL = 35.2535

BMDU = 84.4376

Taken together, (35.2535, 84.4376) is a 90 % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor = 0.0028366

Figure B-8. Renal adenomas or carcinomas, male rats.



Source: Based on data from NTP (1998).

```
====================================================================
        Multistage Cancer Model
====================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Effect
   Independent variable = dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008


                  Default Initial Parameter Values
```

Background = 0.0998638
Beta(1) = 0.000398329

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.7
Beta(1)	-0.7	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0903959	*	*	*
Beta(1)	0.000405707	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-25.0322	4			
Fitted model	-25.0786	2	0.0929231	2	0.9546
Reduced model	-26.8314	1	3.59837	3	0.3082

AIC: 54.1573

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0904	1.085	1.000	12	-0.085
105.0000	0.1283	0.770	1.000	6	0.281
316.0000	0.1998	4.197	4.000	21	-0.107
948.0000	0.3808	4.951	5.000	13	0.028

Chi^2 = 0.10 d.f. = 2 P-value = 0.9520

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 259.696

BMDL = 126.522

BMDU = 2285.4

Taken together, (126.522, 2285.4) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.000790379

APPENDIX C. SUPPLEMENTAL INFORMATION

C.1. OTHER DURATION- OR ENDPOINT-SPECIFIC STUDIES

C.1.1. Acute Toxicity Studies

Oral.

Hofmann and Oettel (1954) examined the effects of THF following oral exposure. Cats (13), rabbits (12), and rats (62, strain and sex not specified) received oral doses (route not specified) ranging from a single administration of 3 cm^3/kg (2,670 mg/kg) to 25 administrations of 1 cm^3/kg (890 mg/kg). The authors reported that no functional or histopathological damage to the liver was observed. Also, no changes were observed in urine analysis, serum urea content, or histopathology of the kidney.

Stasenkova and Kochetkova (1963) evaluated the acute toxicity of THF administered by gavage. White rats (10/group, sex and strain not specified) received THF doses of 1, 1.5, 2, 3, 4, or 5 g/kg by gavage as a solution in 2 mL of distilled water. The rats received a total of six doses, and were observed for clinical signs and mortality. Necropsy and histopathology of major organs was conducted in animals that died during the study exposure period. It does not appear that histopathology was performed on the animals that survived exposure. No mortality was observed at a dose of 2 g/kg. However, a dose of 3 g/kg resulted in 20% mortality, and doses of 4–5 g/kg resulted in 90–100% mortality, respectively. Clinical signs of sedation, including immobility, drowsiness, reduced response to external stimuli, and reduced respiratory rate, were observed after 3–9 minutes of exposure. Mucous membranes appeared to have a cyanotic discoloration. Histopathological lesions were observed in the stomach, brain, liver, heart, spleen, and kidneys and included necrosis, edema, hemorrhage, and excess of blood or fluid in the blood vessels or tissues.

Kimura et al. (1971) investigated the acute oral toxicity of THF in male Sprague Dawley rats (6–12/group). The median lethal dose (LD_{50}) values were estimated for four ages of rats: newborns (24–48 hours old), 14 days old, young adult (80–160 g), and older adult (300–470 g). Single doses of THF (doses unspecified) were administered by gavage; a microsyringe was used for the newborn animals. The oral LD_{50} values for THF were estimated at 2.3 mL/kg for 14-day-old rats, 3.6 mL/kg for young adult rats, and 3.2 mL/kg for older adult rats. The LD_{50} values for the young animals were not statistically different from the values for the older adult rats.

Inhalation.

Stoughton and Robbins (1936) tested the effects of acute inhalation exposure to THF in both mice and dogs. Mice (10/group, strain and sex not specified) were exposed to THF concentrations of 0, 0.5, 1.0, 1.5, 2.2, or 3.0 mmol/L (0, 36,050, 72,100, 108,150, 158,620, or

216,300 mg/m^3) for a single 2-hour exposure. The parameters evaluated included the time required for onset of anesthesia and the time to respiratory failure or death. At the end of the 2-hour exposure, the animals still alive were observed until recovery or death. THF concentrations of 2.2 mmol/L were 100% fatal; at these concentrations, time to onset of anesthesia was 5–8 minutes and time to death was 30–51 minutes. The 1.0 mmol/L dose of THF resulted in 50% mortality, with time to anesthesia of 50 minutes and time to death of 109 minutes. No mortality was observed at a THF concentration of 0.5 mmol/L. Animals surviving at the end of the exposure period regained the ability to walk in 6–8 hours following exposure to THF. One dog (strain and sex not specified) was anesthetized with THF and maintained for 1.5 hours at a THF atmospheric concentration of 5–6%. During this exposure, electroencephalogram (EEG), respiration, and blood pressure were measured. Two days following exposure, the dog was sacrificed and autopsied. Symptoms observed in the dog included increased saliva and mucus flow, decrease in blood pressure, stimulation of respiration, and prolonged sleep up to 6–8 hours after exposure stopped. No gross abnormalities were observed on autopsy.

Henderson and Smith (1936) exposed six rats (strain and sex not specified) to increasing concentrations of THF vapor for 1 hour. The exact concentrations of THF vapor used were not reported, but the authors noted that anesthesia occurred at 6.47% THF. Two animals exposed to just the anesthetic concentration for 30 minutes recovered within 2 minutes after exposure. Two rats that died within 24 hours of exposure had congested, mottled lungs. One rat that initially recovered but appeared ill 4 days later showed fatty changes in the liver.

Hofmann and Oettel (1954) examined the effects of acute inhalation exposure to THF in 18 cats, 20 rabbits, 52 rats, and 150 mice. The sex and strain of the animals were not specified. Animals were exposed to THF vapors at concentrations ranging from 3,400–60,000 cm^3/m^3 (equivalent concentrations reported by the authors were 10,000–193,000 mg/m^3). Exposure regimens ranged from one 2-hour exposure to 30 6-hour exposures. No additional information was provided on exposure durations and concentrations. Therefore, it is not possible to estimate adjusted exposure concentrations. Liver function was assessed by using a bromosulfalein test (decreased clearance of bromosulfalein from the blood is indicative of liver dysfunction). Kidney function was also assessed by urinalysis and serum urea content. Blood cell count was evaluated. Both the liver and kidney were evaluated histopathologically. The authors reported a slight, transient retention of bromosulfalein immediately following exposure to narcotic concentrations of THF.

LaBelle and Brieger (1955) evaluated the effects of acute THF inhalation exposure in rats and mice. Groups of eight male albino rats were exposed to a fixed concentration of THF for a single 4-hour exposure period. Those animals surviving were observed for 14 days. The range of concentrations tested was not specified. This procedure was repeated until the median lethal concentration (LC_{50}) could be determined. In addition, groups of white mice (6/group, sex not specified) were exposed continuously to saturated THF vapor (approximately 47,000 ppm or 138,650 mg/m^3), and survival time was recorded. For mice, the mean survival time following exposure to saturated vapor was 41 minutes. In rats, the LC_{50} reported by the authors was 18,000 ppm (53,100 mg/m^3). Narcosis was reported in rats prior to death.

Stasenkova and Kochetkova (1963) evaluated the effects of a single 2-hour inhalation exposure to THF in white mice and rats (10/group, sex and strain not specified). THF vapor was generated by allowing it to evaporate from a filter paper, so constant air concentrations were not maintained for the duration of the exposure period. For example, at the highest target concentration of 180 mg/L, air concentrations in the test chamber were reported as 140 mg/L after 15 minutes and 65 mg/L after 2 hours. Based on the average of the measurements at 15 minutes and 2 hours, actual mean exposure concentrations were 0, 7, 13, 19, 27, 42, 73, 80, and 103 mg/L (0, 7,000, 13,000, 19,000, 27,000, 42,000, 73,000, 80,000, and 103,000 mg/m^3). Animals were evaluated for clinical signs and mortality. Histopathological examination was conducted on animals that died. The authors did not indicate whether histopathological examinations were conducted on the animals that survived exposure. In mice, the average concentration of 19 mg/L resulted in 80% mortality, and 27 mg/L resulted in 100% mortality. Rats were less sensitive to THF. The average concentration of 42 mg/L resulted in 20% mortality, and 80 mg/L resulted in 100% mortality. The animals displayed symptoms of sedation and narcosis, including depressed activity, interrupted breathing, and reduced coordination of movement. In addition, mucus membranes were pale and bluish in color. Lesions observed in lungs and bronchi included excess blood or fluid, edema, perivascular hemorrhage, and catarrhal condition of the mucus membrane. Histopathological lesions were also observed in brain, liver, kidney, and spleen, including excess blood or fluid, edema, and dystrophic changes.

DuPont Haskell Laboratory (1979) conducted an acute inhalation study of THF in order to determine the highest concentration of THF that would not produce narcosis in rats. ChR-CD rats (6/sex/group) were exposed to THF concentrations, ranging from 3,010–20,500 ppm (8,880–60,475 mg/m^3) for a single 6-hour exposure period. Following exposure, all rats were weighed daily and clinical signs were observed for 14 days. The authors determined that the non-narcotic concentration in male rats was 5,380 ppm (15,871 mg/m^3) and in female rats was 5,700 ppm (16,815 mg/m^3). During the exposure period, both male and female rats demonstrated clinical signs of pawing and scratching and decreased or no response to sound at all concentrations. Male rats also exhibited signs of rapid respiration, and females showed signs of paralysis. Based on clinical signs of CNS toxicity, the lowest exposure concentration of 8,880 mg/m^3 was the study LOAEL.

Ohashi et al. (1983) evaluated the effects of acute inhalation exposure to THF on the upper respiratory tract (nasal mucosa) of rabbits. Adult rabbits (sex and number not specified) were exposed to THF concentrations of 100, 250, 1,000, 2,000, 6,000, or 12,000 ppm (295, 738, 2,950, 5,900, 17,770, or 35,400 mg/m^3) for a single 4-hour exposure period. The rabbits were sacrificed by air embolization, and their nasal mucus membranes were obtained at 0, 20, 40, 60, 120, or 180 minutes following exposure. The membranes were evaluated for ciliary beating frequency and examined by scanning electron microscopy. No other organs or systems were evaluated. THF caused a dose-related decrease in ciliary beating frequency. Concentrations of 250 ppm caused about a 50% decrease in beat frequency that returned to normal within 3 hours following exposure. Concentrations of 1,000 ppm almost completely eliminated ciliary beating, and at these concentrations beat activity did not return to normal. THF concentrations of 250 ppm resulted in the appearance of sporadic compound cilia, but no other morphological changes. Concentrations of 1,000, 2,000, and 6,000 ppm resulted in the increased incidence of compound cilia and the vacuolation of epithelial cells, indicating moderate degeneration. At 12,000 ppm THF, observations included many large compound cilia, vacuolation, cytoplasmic protuberances, and sloughing of the epithelial cells, indicating severe degeneration. Based on significant morphological changes to nasal epithelial cells, 1,000 ppm (2,950 mg/m^3) was the study LOAEL and 250 ppm (738 mg/m^3) was the study NOAEL.

Horiguchi et al. (1984) evaluated the acute toxicity of THF following inhalation exposure in rats. Sprague-Dawley rats (6 males/group) received a single 3-hour exposure to THF at concentrations of 200, 1,000, 5,000, 10,000, 15,000, 25,000, or 30,000 ppm (590, 2,950, 14,750, 29,500, 44,250, 73,750, or 88,500 mg/m^3). The animals were observed for clinical signs of toxicity, abnormal behavior, and mortality for 72 hours following exposure. The LC_{50} value was estimated to be 21,000 ppm (61,950 mg/m^3) by using a probit method. Animals in the 200 ppm group displayed signs of head shaking and face washing, as well as patches of mild irritation on nose, ears, and eyelids, and tended to sleep. Symptoms of irritation increased with the exposure concentration. At 5,000 ppm, animals displayed intense salivation, tearing, and bleeding from the nose. In addition, animals developed clonic muscle spasms, had altered respiratory patterns, and became comatose about 1 hour following the start of exposure. All animals in the 25,000 ppm group died within 72 hours following exposure. No information was provided regarding the observations in other dose groups. Based on clinical signs of irritation and neurotoxicity, the concentration of 5,000 ppm (14,750 mg/m^3) was the LOAEL in this study.

Ikeoka et al. (1988) investigated the effects of acute inhalation exposure to THF on the lower respiratory tract (tracheal mucosa) of rabbits as a follow-up to the earlier study by Ohashi et al. (1983). Adult rabbits (sex and number not specified) were exposed to THF at concentrations of 100, 250, 1,000, 2,000, 6,000, or 12,000 ppm (295, 738, 2,950, 5,900, 17,770, or 35,400 mg/m^3) for a single 4-hour exposure period. The authors did not state if a control group was also included. The rabbits were sacrificed by air embolization, and their tracheal mucosa membranes were obtained at 0, 20, 40, 60, 120, or 180 minutes following exposure. The membranes were evaluated for ciliary beating frequency and examined by scanning electron microscopy. No other organs or systems were evaluated. THF caused a dose-related decrease in ciliary beating frequency. Concentrations of 250 ppm caused about a 50% decrease in beat frequency that returned to normal within 3 hours following exposure. Concentrations of 1,000 ppm almost completely eliminated ciliary beating, and at these concentrations beat activity did not return to normal within 3 hours. Compound cilia, ballooning, and vacuolation of tracheal epithelial cells were observed in the high-concentration group. However, the areas of severe degeneration observed in the nasal epithelium following the same exposure protocol were not observed in the trachea in the current study. The effects on the tracheal morphology were mild compared with those observed in nasal epithelium by Ohashi et al. (1983). Based on tracheal histopathology, 12,000 ppm (35,400 mg/m^3) was the study LOAEL and 6,000 ppm (17,770 mg/m^3) was the study NOAEL.

Dermal.

Stasenkova and Kochetkova (1963) evaluated the effects of THF application to the skin of white mice (20, strain and sex not specified) and rabbits (number, sex, and strain not specified). Pure THF (1 mL) was applied to the skin of rabbits. THF caused reddening of the skin, which subsequently thickened and sloughed off. Pure THF applied to the eyes of rabbits caused edema of the eyelid, vasodilation, and corneal opacity. The tails of mice were immersed in pure THF for 2 hours. This treatment resulted in mortality, symptoms typical of THF poisoning, as well as excess blood or fluid and hemorrhage of internal organs.

C.1.2. Short-term Studies

Oral.

Komsta et al. (1988) reported the results of a short-term oral toxicity study of THF in rats. Sprague-Dawley rats (10/sex/group) were administered THF in drinking water at concentrations of 0, 1, 10, 100, or 1,000 mg/L for 4 weeks. The equivalent doses estimated by the study authors based on measured water consumption and body weights were 0, 0.1, 0.8, 10.2, and 95.5 mg/kg-day. Clinical signs, body weight gain, and food and water consumption were evaluated weekly. Following the exposure period, the animals were sacrificed and examined at gross necropsy. Organ weights were obtained for brain, heart, liver, spleen, and kidney. Blood was collected for hematology and serum chemistry evaluation. A selection of tissues from the control and high-dose group was evaluated histopathologically.

There was no increase in mortality in any of the dose groups, and no clinical signs were observed in any of the treated animals. In addition, body weight gain and food and water consumption were not significantly different between treated and control animals. No changes in hematology or serum chemistry were observed in treated animals. Some sporadic observations of histopathological changes were observed in the thyroid, liver, and kidney; however, the incidence for these findings was comparable in treated and control animals. Male rats in the high-dose group demonstrated a higher incidence of increased cytoplasmic homogeneity in liver compared with controls (3/10 and 7/10 for control and high-dose animals, respectively). No changes in any of the biochemical parameters evaluated were observed. Female rats showed an increased incidence of anisokaryosis (unequal size of cell nuclei) in the liver (0/10 and 7/10 for control and high-dose animals, respectively) and tubular cytoplasmic inclusions in the kidney (0/10 and 3/10 for control and high-dose animals, respectively). The authors did not conduct a statistical analysis of the incidence data. In addition, histopathology was not performed on the lower dose groups, so it is not possible to evaluate the dose-response relationship for these endpoints. The study authors concluded that THF in drinking water at doses up to 1,000 mg/L did not produce overt toxicity. Komsta et al. (1988) also indicated that the effects observed at the high dose of THF were considered mild and adaptive and could not be related to any functional changes (i.e., altered biochemical parameters).

Pozdnyakova (1965) evaluated the effects of short-term exposure to THF in drinking water. White mice (number, sex, and strain not specified) received THF in the drinking water at concentrations of 40 and 100 mg/L for 45 days. Mice in the high-dose group exhibited decreased body weight, paralysis of hind legs, leukocytosis, and decreased hemoglobin. No significant changes were observed in the low-dose group. No additional information was provided about the study.

In the same study report, Pozdnyakova (1965) exposed 20 rabbits (sex and strain not specified) and 50 white rats (sex and strain not specified) to THF in drinking water at doses of 10 and 20 mg/kg. The study was classified as being chronic in duration by the study authors; however, the actual duration of exposure was not specified. Rabbits in the high-dose group exhibited a change in cholinesterase activity, an increase in prothrombin time, and a low serum antibody titer compared with controls. Rats in the high-dose group showed a reduction in body weight and a change in serum albumin content. No additional information was provided in the study.

Inhalation.

Horiguchi et al. (1984) evaluated the ability of THF to irritate the respiratory tract following short-term inhalation exposure to THF. Male Sprague-Dawley rats (3–6/group) were exposed to 0, 100, or 5,000 ppm (0, 295, or 14,750 mg/m^3) THF vapor for up to 3 weeks. No information was provided on the duration of each exposure period or the number of days/week the animals were exposed, and therefore duration-adjusted exposure concentrations could not be calculated. A single animal was randomly selected from each exposure group 1 day, 1 week, and 3 weeks following the start of exposure. The animals were sacrificed the next day (24 hours later) and the respiratory tract mucous membrane was extracted and prepared for histological examination. No differences were observed between the tracheal mucosa of the treated groups and the controls following 1 day or 1 week of exposure. By 3 weeks of exposure, the tracheal mucosa of animals in the high-concentration group exhibited disordered cilia and epithelial cells and darkening of cell bodies compared with control animals. Also, by 3 weeks of exposure, the nasal mucosa of animals in the low-concentration group (100 ppm) exhibited the same type of changes described above for the tracheal mucosa (e.g., disordered cilia and epithelial cells and darkening of cell bodies) without significant histopathological effects. The nasal mucosa of animals exposed to 5,000 ppm for either 1 week or 3 weeks, however, demonstrated disruption of the epithelial architecture, congestion, and sloughing of ciliary and goblet cells, in addition to vacuolation and darkening of cell bodies. Based on these effects at the nasal mucosa, the study LOAEL was determined to be 5,000 ppm and the study NOAEL was 100 ppm.

Stasenkova and Kochetkova (1963) evaluated the short-term effects of THF inhalation in male rats and mice (20/group; strain not specified). The animals were exposed for 2-hour periods, twice a day, every day for 2 months to air concentrations of THF ranging from 6 to 8 mg/L (6,000–8,000 mg/m^3). However, THF vapor was generated by allowing it to evaporate from a filter paper, so constant air concentrations were not maintained for the duration of each exposure period. Animals were evaluated for clinical signs, mortality, and body weight. Endpoints evaluated included the threshold of neuromuscular irritability (method of measurement was not specified), arterial blood pressure, blood cell counts, liver function (measured by synthetic capacity), and kidney function (measured by albumin in urine). After 2 months, the animals were sacrificed and histopathological examination of major organs was conducted.

All animals developed symptoms of narcosis during the exposure; however, this effect was not observed during the periods between exposures. By day 40 of exposure, treated rats had reduced body weight compared with controls. At the end of the 2-month study period, mean body weights of treated rats was 30% less than controls. In addition, treated rats had a lower threshold of neuromuscular irritability than controls. No effects in rats were observed on blood pressure, blood cell count, or liver or kidney function. Histopathological lesions in the respiratory tract included catarrhal rhinitis, bronchitis, proliferative reaction in lungs, emphysema, and hypertrophy of muscle fibers in the walls of the bronchi. Histopathological lesions, including hypertrophy of muscle fibers and perivascular sclerosis, were observed in the heart, liver, and kidneys. Incidence data were not provided for any of these histopathological findings.

Treated mice initially developed symptoms of eye and respiratory tract irritation and had an increase in the threshold of neuromuscular irritability compared with controls. After 1 month of treatment, mortality in mice increased. The authors indicated that mice died of bronchial pneumonia. It was not clear if mortality in controls was increased and if the bronchial pneumonia was a cause of THF treatment or a bacterial infection in the mice. The mice still living at the end of the 2-month treatment period had a 15–20% decrease in body weight compared with controls. No information was provided on the results of other endpoints evaluated in mice. Because of poor reporting of this study, no NOAEL−LOAEL was determined.

C.1.3. Neurotoxicity Studies

DuPont Haskell Laboratory (1996a), published in the peer-reviewed literature as Malley et al. (2001), investigated the neurotoxicity of acute inhalation exposure to THF in rats. Crl:CD BR rats (12/sex/group) were exposed to THF vapor at concentrations of 0, 500, 2,500, or 5,000 ppm (0, 1,475, 7,375, or 14,750 mg/m^3) for a single 6-hour exposure (designated as test day 1). The animals were then observed for 2 weeks following exposure. Clinical signs, body weight, and food consumption were evaluated weekly. The response to an alerting stimulus was determined as a group for each exposure concentration, prior to the start of exposure and approximately 2 and 4 hours after initiation of exposure. All rats were evaluated for neurobehavioral effects. Motor activity assessments and functional observational battery (FOB) assessments were conducted before exposure and on test days 2, 8, and 15. For the motor activity assessments, animals were individually tested in an automated activity monitor that measured both duration of continuous movements and number of movements. The FOB assessment consisted of a series of quantified behavioral evaluations conducted in a sequence that proceeded from the least interactive to the most interactive. During the FOB assessment, each rat was evaluated in three environments: inside the home cage, on removal from the home cage while being handled, and in a standard open field arena.

Exposure to 2,500 ppm THF appeared to have an effect on response to alerting stimulus in rats. Six of 24 rats in the 2,500 ppm group had a diminished response after 2 hours of exposure, and all 24 rats in this group had diminished response after 4 hours of exposure. Half the rats in the 5,000 ppm group had diminished response after 2 hours of exposure, and all of the rats had either no response or diminished response to stimulus after 4 hours of exposure. Other signs of sedation in the high concentration group included a significant increase in the incidence of lethargy and abnormal gait in both male and female rats at 5,000 ppm. Male rats in the 5,000 ppm group had significantly decreased body weight gain and food consumption in the interval between test day 1 and 2, although these values were comparable to controls for the remainder of the observation period. Several parameters in the FOB were affected in the 5,000 ppm groups immediately following the exposure period only, including the righting reflex in males and females, palpebral closure in females, and ease of handling in females. The effects on FOB parameters were not observed during test days 2, 8, or 15, suggesting that the sedative effects of THF were short-lived. The LOAEL for this study is 2,500 ppm (7,375 mg/m^3), based on observations of sedative effects, and the NOAEL for this study was 500 ppm (1,475 mg/m^3).

DuPont Haskell Laboratory (1996b; Malley et al., 2001) investigated neurotoxicity following subchronic inhalation exposure to THF in rats. Crl:CD BR rats (12–18/sex/group) were exposed to THF vapor at concentrations of 0, 500, 1,500, or 3,000 ppm (0, 1,475, 4,425, or 8,850 mg/m^3) 6 hours/day, 5 days/week over a 13- to 14-week exposure period. Clinical signs, body weight, and food consumption were evaluated weekly. Prior to the start of exposure and approximately 2, 4, and 6 hours after initiation of exposure, the response to an alerting stimulus was determined for the rats as a group for each exposure concentration. All rats were evaluated for neurobehavioral effects. Motor activity assessments and FOB assessments were conducted before the first exposure and at 4, 8, and 13 weeks. For the motor activity assessments, animals were individually tested in an automated activity monitor that measured both duration of continuous movements and number of movements. The FOB assessment consisted of a series of quantified behavioral evaluations conducted in a sequence that proceeded from the least interactive to the most interactive. During the FOB assessment, each rat was evaluated in three environments: inside the home cage, after removal from the home cage while being handled, and in a standard open field arena. Rats (6/sex/group) were sacrificed after 13 weeks of exposure, and tissue from the nervous system and muscle was assessed histopathologically.

The only effects observed in this study appeared to be related to the acute sedative effects of THF characterized by the study authors as acute behavioral sedation, which dissipates rapidly upon termination of exposure (Malley et al., 2001). A diminished response to alerting stimulus during exposure was observed in male and female rats in the 1,500 and 3,000 ppm exposure groups. In the 3,000 ppm group, diminished response was observed consistently, beginning on the second day of exposure. In the 1,500 ppm group, diminished response was observed sporadically from days 16 to 49 of exposure and observed consistently on the remaining exposure days. Diminished response was observed sporadically from days 16 to 49 of exposure and observed consistently on the remaining exposure days. Compound-related clinical signs, including stained nose and stained/wet perineum, were also observed in male and female rats in the 1,500 and 3,000 ppm groups. These signs were not observed on Mondays prior to the start of exposure for the week or on the days of the motor activity and FOB assessment. Therefore, these signs were considered to be transient. No effects were observed on body weight, body weight gain, food consumption, motor activity, any of the parameters in the FOB, or neuropathology in either male or female rats at any concentration. Based on clinical signs of sedation during exposure to THF, 1,500 ppm (4,425 mg/m^3) was the study LOAEL, and the NOAEL for this study was 500 ppm (1,475 mg/m^3). However, the authors suggested that these effects were transient.

Marcus et al. (1976) evaluated the neuropharmacological effects of THF administered by i.p. injection. Male Sprague-Dawley rats (number/group not specified) were implanted with electrodes to facilitate continuous EEG recordings. THF was administered by i.p. injection at doses of 15 and 21 mmol/kg (1,156 and 1,619 mg/kg). After a 2-minute latency period, 21 mmol/kg THF induced high amplitude slow wave activity in the EEG, which lasted 2 minutes. The EEG pattern then changed to spiking and electrical silence, which lasted for 20 minutes. The altered EEG pattern was accompanied by loss of the righting reflex. A dose of 15 mmol/kg induced a desynchronization of the EEG activity without loss of the righting reflex.

In an in vitro study, THF caused a decrease in adenosine triphosphatase (ATPase) activity and membrane fluidity in a dose-dependent manner in an assay using rat brain synaptosomes (Edelfors and Ravn-Jonsen, 1992).

C.2. METABOLITE AND MECHANISTIC DATA AND OTHER STUDIES

C.2.1. Metabolite Studies

The nervous system is one of the primary targets of THF toxicity. As discussed under Metabolism (Section 3.3), the effects of THF on the nervous system may be due to its metabolites, GBL and GHB. Major study findings of these compounds are briefly summarized (Table C-1) to facilitate an evaluation of THF toxicity data, but a more detailed review is available (NSF, 2003).

Table C-1. Comparison of target organ toxicity for THF and its metabolites

Target organ	THF	GBL	GHB
CNS	No effect in rat drinking water study at 882 mg/kg-day. Narcosis observed in inhalation studies at estimated systemic doses of 2,260 mg/kg-day in mice[a] and 5,822 mg/kg-day in rats[b].	Lethargy in rat and mice subchronic gavage at 225 mg/kg-day (NTP, 1992); EEG changes beginning at 50 mg/kg i.p. in young rats in mode of action studies (Takizawa et al., 2003)	Dizziness in human clinical studies at 12.5 mg/kg LOAEL (Ferrara et al., 1999)
Liver	No effect in rat drinking water study at 788 mg/kg-day. Increased absolute and relative liver weight in mice in the inhalation study at estimated systemic dose of 753 mg/kg-day.	No effect in subchronic gavage study at 900 mg/kg-day (rats) and 1,050 mg/kg-day (mice) (NTP, 1992)	No data
Kidney	Increased kidney weight in rat drinking water study at 714 mg/kg-day.	No effect in subchronic gavage study at 900 mg/kg-day (rats) and 1,050 mg/kg-day (mice) (NTP, 1992)	No data
Thymus	No oral data. Decreased thymus weight at 753 mg/kg-day and thymus atrophy at 2,260 mg/kg-day in mouse inhalation study.	Thymus depletion at 262 mg/kg-day in mouse 2-year gavage study[c] (NTP, 1992)	No data
BW	Minimally decreased body weight in rat drinking water study at 714 mg/kg-day.	Decreased body weight in rat 2-year gavage study at 450 mg/kg-day and in mice at 262 mg/kg-day (NTP, 1992)	No data
Development	Decreased pup body weight gain, delayed eye opening, and increased incidence of sloped incisors at 782 mg/kg-day in rat drinking water study. Fetal weight, skeletal alterations in rat inhalation studies.	No effects in rat gavage at 500 mg/kg-day (Kronevi et al., 1988)	No data
Reproductive	No effect in rat drinking water study on reproductive function or testes weight at 788 mg/kg-day.	Decreased testes weight in rat gavage study at LOAEL of 667 mg/kg-day (Debeljuk et al., 1983)	No data

[a]For this cursory analysis, estimated systemic doses were calculated from the inhalation studies assuming 100% absorption and EPA default parameter values for mice as follows: lowest-observed-effect level (LOEL) exposure concentration (mg/m^3) × default EPA ventilation rate (0.063 m^3/day) × study exposure duration (6 hours/24 hours)/default EPA BW (0.037 kg) = mg/kg-day.
[b]For this cursory analysis, estimated systemic doses were calculated from the inhalation studies assuming 100%absorption and EPA default parameter values for rats as follows: LOEL exposure concentration (mg/m^3) × default EPA ventilation rate (0.36 m^3/day) × study exposure duration (6 hours/24 hours)/default EPA BW (0.38 kg) = mg/kg-day.
[c]No effects on thymus weight were observed in the 13-week study (NTP, 1992). Thymus histopathology in the chronic study (NTP, 1992) was attributed by the authors to injuries secondary to fighting.

There is no specific organ toxicity information following repeated human exposure to GBL; however, chronic use of GBL as a drug of abuse can lead to neurotoxicity, including addiction, anxiety, depression, insomnia, and tremors (Herold and Sneed, 2002). The systemic toxicity of GBL has been investigated in a full 2-year bioassay in rats and mice that employed gavage dosing (NTP, 1992). The most sensitive effect observed in these studies was clinical signs of CNS toxicity (lethargy) with a NOAEL of 112 mg/kg-day in rats. The only other treatment-related effect observed in rats and mice was for decreased body weight. NTP (1992) also reported a statistically significant increase in the incidences of thymic depletion and epithelial hyperplasia of the thymus in the mid- and high-dose male mice (0/42, 5/39, and 6/38 and 0/42, 4/39, and 4/38, respectively). The study authors concluded that the observed dose-related increase in these non-neoplastic lesions was related to fighting in the male mice. Specifically, the depletion of lymphocytes in the thymus (often seen with debilitation and stress in animals) was most often observed in mice dying early as a result of wounds received from fighting. The relevance of the observed effects on the thymus remains uncertain.

In other studies on GBL, no prenatal developmental effects were observed in rats at doses up to 500 mg/kg-day (Kronevi et al., 1988), while decreased testicular weight was reported in a short-term reproductive study (Debeljuk et al., 1983) with a LOAEL of 667 mg/kg-day.

The oral toxicity data for GHB are primarily from clinical studies in human subjects or from case reports of oral poisonings. Transient dizziness and a sense of dullness in 50% of human subjects following a single oral dose of 12.5 mg/kg were observed by Ferrara et al. (1999). Standardized measure of psychomotor performance was not affected at this dose (Ferrara et al., 1999). Metcalf et al. (1966) reported the effects of single oral doses of 35–63 mg/kg GHB in volunteers. All participants reported drowsiness during the experiment and some participants receiving doses >50 mg/kg were rendered unconscious. Medical anesthetic doses of GHB are typically in the range of 60 mg/kg (Miotto et al., 2001; Vickers, 1969; Root, 1965).

In the case of GHB, the dosing regimen seems to play an important role on the induction of CNS effects. The human clinical studies make it clear that for the CNS effects of GHB, bolus dosing regimens have an important effect. For example, as shown in Table C-2, large differences in total daily dose did not show a significant change in overall response rate and severity when the individual doses were similar (Gallimberti et al., 1993, 1992). Furthermore, the incidence of effects and their severity generally corresponds to the individual doses rather than the total daily dose (Nimmerrichter et al., 2002; Gallimberti et al., 1993).

Table C-2. Comparative effects of single and multiple daily dosing of GHB

Reference	Single dose (mg/kg)	Maximum total daily dose (mg/kg-day)	Effect
Gallimberti et al. (1993)	25	300	Dizziness (5/41)
Gallimberti et al. (1992)	17	50	Dizziness (3/41)
Addolorato et al. (1998)	50	150	Vertigo and lethargy (30% of 109 patients)
Nimmerrichter et al. (2002)	10–20 20–40	50 100	Vertigo (9/31); majority after the double dose Vertigo (17/33); seizure (1/33); disorientation (1/33)—majority after the double dose
Scharf et al. (1998)	30	60	Altered brain wave measurements during sleep

Peak doses rather than cumulative doses appear to drive the CNS response to administration of GHB. The absence of observed CNS effects in the two-generation THF drinking water study in rats (Hellwig et al., 2002; BASF, 1996) at higher daily doses than the daily gavage doses for GBL, which also caused CNS effects (NTP, 1992), may reasonably be explained by differences in exposure patterns. Continuous drinking water exposures might not result in sufficient peak levels of exposure to induce the CNS effect. Other explanations may exist for the absence of reported CNS effects in the two-generation study including, for instance, lack of a more detailed neurobehavioral evaluation and other limitations in study design including lack of sensitivity or suitability for analyzing neurotoxicity potential.

C.2.2. Mechanistic Studies

C.2.2.1. *Cytotoxicity*

THF was evaluated in a series of short-term in vitro assays to assess its potential for cytotoxicity (Curvall et al., 1984): inhibition of cell growth of ascites sarcoma BP 8 cells grown as stationary suspension cultures, inhibition of oxidative metabolism in isolated brown fat cells, plasma membrane damage (leakage of a cytoplasmic nucleotide marker from prelabeled cells), and ciliotoxicity as measured by time to ciliostasis in cultures of trachea from unborn chickens. To facilitate comparison of multiple chemicals, the results from each individual assay were expressed as a percentage of control responses and then these percentages were converted to a 10-point scale where 0 corresponded to 0–9%. The response observed in each of the individual assays of THF was <10%. THF was scored 0 for each of the individual assays and for its mean cytotoxicity activity. In contrast, several chemicals, mostly alkylphenols, were highly active in the test systems, having activity of 7 in each of the test systems. In a related study, a 5 mM concentration of THF took >60 minutes to cause ciliostasis in an in vitro assay in embryonic chicken trachea, whereas highly cytotoxic compounds caused ciliostasis in <5 minutes (Pettersson et al., 1982). Therefore, the results of these studies suggest that THF is not cytotoxic.

The cytotoxicity of THF was evaluated in an in vitro assay of protein content in cell cultures (Dierickx, 1989). Human hepatoma, HepG2 cells were maintained in culture in 24 well tissue culture test plates. THF and other test compounds were dissolved directly in culture medium at five different concentrations (not specified) and incubated with test cells for 24 hours. The cells were lysed and protein content measured. The relative toxicity of THF and the other test compounds was determined by estimating the concentration (in mM) required to induce a 50% reduction of cell protein content (PI_{50}). Very toxic compounds, such as heavy metals and surfactants, consistently had PI_{50} values of less than 1 mM. In contrast, the PI_{50} for THF was 372. The results of this study suggest that THF is not cytotoxic.

The cytotoxicity of 168 chemicals, including THF, was characterized as part of a validation of the BALB/c-3T3 cell transformation assay (Matthews et al., 1993). The LC_{50} for THF was 90.3 mM. The authors noted that in the analysis of the entire data set of 168 chemicals, in vitro cytotoxicity did not correlate to in vivo carcinogenic activity. THF was considered by the authors as noncytotoxic (defined as having an LC_{50} ranging from 5 to 100 mM).

C.2.2.2. *CYP450 Activity, Cell Proliferation, and Apoptosis*

BASF (1998) re-evaluated kidney tissues from male rats and liver tissues from female mice from the NTP (1998) study to examine the relationship between cell proliferation responses and increase in tumors observed in these tissues following THF administration. Histopathological examination and evaluation of cell proliferation as measured by proliferating cell nuclear antigen (PCNA) staining were conducted using tissue samples from the 0, 200, 600, and 1,800 ppm (0, 590, 1,770, and 5,310 mg/m^3) exposure groups (10/group) from the NTP (1998) subchronic (13 weeks) study. For the male rat kidneys, tissues from the cortex, outer stripe of the outer medulla, inner stripe of the outer medulla, and inner medulla were evaluated separately. For the female mouse liver, no zonal subdivision was made.

The histopathology examination revealed increased incidence of moderate grade hyaline droplet accumulation in the male rat kidney tissues of the high-concentration group as compared to controls, but these changes were not accompanied by evidence of cell degeneration. No other differences between controls and exposure groups were noted. No increase in cell proliferation was found in any of the kidney compartments or in evaluation of all compartments combined. Cell proliferation index was statistically significantly decreased in individual kidney compartments, although these changes did not show a concentration-dependent pattern. For the female mouse liver tissues, no treatment-related histopathology was observed. The cell proliferation index was increased by approximately 39% in tissues from the high-concentration mice compared with controls. However, this result was not statistically significant and was noted as being predominantly based on the results from 2/10 animals. Furthermore, a significant decrease in proliferation index was observed in the mid-concentration group, but no clear concentration-response pattern was observed. Based on these results, the study authors concluded that the examination of the tissues from the 13-week NTP (1998) study revealed no clear increase in cell proliferation that can be correlated to a tumorigenic mechanism.

BASF (Gamer et al., 2002; BASF, 2001a) evaluated a series of endpoints in male F344 rats (6/group plus 5/group at the control and high concentrations for enzyme assays) and female $B6C3F_1$ mice (10/group plus 5 in the control and high concentrations for enzyme assays) in tissues for which THF-treated animals developed tumors. Animals were placed in one of three groups that were exposed 6 hours/day for either 5 consecutive days, 5 consecutive days followed by a 21-day observation period, or 20 consecutive days over a period of approximately 28 days. Test animals were exposed nose only to 0, 199, 604, or 1,794 ppm THF (average THF concentrations of 0, 598, 1,811, or 5,382 mg/m^3), corresponding to the concentrations used in the NTP (1998) cancer bioassay. Concentrations adjusted for continuous exposure were 0, 107, 323, or 961 mg/m^3. For the animals in each of the four concentration groups, a full necropsy was done, including histopathological evaluation of the kidney (rat) and liver (mouse). Additional evaluations in these same organs included measurements of cell proliferation (S-phase response by 5-bromo-2-deoxyuridine [BrdU] staining) and terminal deoxynucleotidyl transferase deoxyuridine triphosphate (dUTP) nick-end-labeling staining (TUNEL) apoptosis assay. For the male rat kidneys, immunohistochemical detection of α_{2u}-globulin was also performed. Five animals from the control and high-concentration groups that were exposed for 5 consecutive days were also harvested for measurement of CYP450 content and for CYP450 isozyme activity as measured by ethoxyresorufin-O-deethylase (EROD) and pentoxyresorufin-O-depentylase (PROD) activity.

The results of the BASF (2001a) study, evaluating cell proliferation, apoptosis, and α_{2u}-globulin accumulation in the kidneys of male F344 rats, are shown in Table C-3. Although no significant increase in labeling index in the renal cortex was determined by standard assessment methods, focal areas of increased BrdU labeling were noted. Quantitation of these areas revealed increased cell proliferation in subcapsular proximal tubules (cortex 1) in animals exposed to THF at the mid and high concentration for 20 days and at the high concentration for 5 consecutive days. No increase in labeling was observed in the groups given a 21-day recovery period. An increase in cell proliferation was also noted in the proximal tubules between the outer stripe of the outer medulla and the subcapsular layer (cortex 2) at the highest concentration following 20 exposures.

Table C-3. Mode of action study findings in male F344 rat kidneys following exposure to THF by inhalation

Exposure protocol	Control		600 mg/m^3		1,800 mg/m^3		5,400 mg/m^3	
	%	LC (M)[a]	%	LC (M)	%	LC (M)	%	LC (M)
5 Exposures								
BrdU labeling: cortex 1	100	112	95	107	109	122	153[b]	171
BrdU labeling: cortex 2	100	132	102	134	99	131	125	165
TUNEL: whole cortex	100	13[c]	115	15	107	14	92	12
5 Exposures + 3 weeks recovery								
BrdU labeling: cortex 1	100	138	78[d]	107	88	121	110	152
BrdU labeling: cortex 2	100	140	86	121	86	120	105	147
TUNEL: whole cortex	100	9	45	4	145	13	478[b]	43
20 Exposures								
BrdU labeling: cortex 1	100	118	119	140	159[b]	188	298[b]	352
BrdU labeling: cortex 2	100	156	101	158	113	176	186[b]	290
TUNEL: whole cortex	100	35	74	26	157	55	234[b]	82
	Control		600 mg/m^3		1,800 mg/m^3		5,400 mg/m^3	
Exposure protocol	%	LA (%)	%	LA (%)	%	LA (%)	%	LA (%)
5 Exposures								
α_{2u}-globulin: whole cortex	100	6.16	136[d]	8.37	171[b]	10.53	178[b]	10.95
α_{2u}-globulin: cortex 1	100	7.30	125	9.14	167[b]	12.18	175[d]	12.75
α_{2u}-globulin: cortex 2	100	5.01	131	6.57	176[b]	8.82	188[b]	9.42
5 Exposures + 3 weeks recovery								
α_{2u}-globulin: whole cortex	100	5.57	150	8.35	212[b]	11.80	299[b]	16.66
α_{2u}-globulin: cortex 1	100	6.68	154	10.32	213[b]	14.22	280[b]	18.70
α_{2u}-globulin: cortex 2	100	4.47	141	6.30	205[d]	9.18	324[b]	14.49
20 Exposures								
α_{2u}-globulin: whole cortex	100	5.34	149[d]	7.97	221[b]	11.79	259[b]	13.84
α_{2u}-globulin: cortex 1	100	6.20	149[b]	9.21	212[b]	13.15	253[b]	15.70
α_{2u}-globulin: cortex 2	100	4.47	149[d]	6.66	236[b]	10.53	265[b]	11.86

[a]LC (M) = positively labeled cells (LCs) mean value.
[b]$p \leq 0.01$.
[c]Number of apoptotic cells.
[d]$p \leq 0.05$.

Source: Adapted from BASF (2001a).

To determine whether changes in cell proliferation might reflect altered apoptosis rates, apoptotic cells were also quantified (Table C-3). The number of cells undergoing apoptosis was significantly increased in the high-concentration groups exposed for 5 days and observed for 21 days or after 20 exposure days. Marginal increases were observed in the mid-concentration groups for these two exposure regimens, but the results were not statistically significant. The authors suggested the increase in apoptosis observed in the group with a recovery period might be greater than in the 20-day exposure group, because in the latter group competing cell proliferation and apoptosis events might have reduced the degree of apoptosis.

THF exposure also induced α_{2u}-globulin accumulation in male rats treated under all three of the separate exposure regimens (Table C-3). Increases were generally concentration related, with increases at the high concentration ranging from 175 to 280% of control levels for cortex 1 and from 188 to 324% of control levels for cortex 2, among the three exposure regimens. When the whole cortex was used as the labeled area (LA) for the analysis, accumulation was significantly elevated beginning at the low concentration following 5 consecutive days or 20 days of exposure. Maximum effects observed at the high concentration ranged from 178 to 299% of controls among the three exposure regimens. The accumulation of α_{2u}-globulin as measured by the immunohistochemical staining technique was supported by histopathological evaluation of control and high-concentration animals exposed to THF for 20 days. The incidence of proximal tubule cells with grade 2 (slightly increased) staining for hyaline droplets was 1/6 and 5/6 for controls and high-concentration animals, respectively. THF exposure had no effect on CYP450 content or CYP450 enzyme activities in the male rat kidneys.

BASF (2001a) and Gamer et al., (2002) also evaluated cell proliferation in female $B6C3F_1$ mice liver following inhalation exposure to THF (Table C-4). Since chemical exposures can have varying effects in different regions of the liver lobule, cell proliferation was evaluated separately for zone 1 (the region adjacent to the portal triad), zone 3 (the region adjacent to the central vein), and zone 2 (the area of the lobule intermediate between zones 1 and 3). Increased cell proliferation was observed in zones 2 and 3 of the liver following THF exposure for 5 days and in zone 3 following 20 exposures. No concentration-dependent increase in BrdU labeling was observed in the animals given a 21-day recovery period, suggesting that the increases in cell proliferation may be reversible. Coincident with the increase in BrdU labeling, the mitotic index (MI) was increased in zone 3 after 5 or 20 exposures in the high-concentration groups. No treatment-related change in the number of liver cells undergoing apoptosis was observed. The number of stained cells was small, suggesting that THF did not induce an apoptotic response under the exposure conditions. Five consecutive days of exposure to THF at the high concentration generated a statistically significant increase in CYP450 content in the liver (125% of controls; $p \leq 0.05$), EROD activity (192% of controls; p 0.01), and PROD activity (321% of controls; $p \leq 0.05$). The authors concluded that THF-induced liver tumors in female mice may be related to increased cell proliferation, based on the increased liver weight, BrdU labeling, and MI observed in the liver. Some histological changes were noted, including fatty infiltration and cell proliferation including altered texture of the cytoplasm in zones 3 and 2 (more homogeneous and eosinophilic); however, no morphological signs of cell degeneration, such as cloudy swelling, vacuolar degeneration, or necrosis were found.

Table C-4. BrdU labeling and MI as a measure of cell proliferation in female B6C3F_1 mouse livers following exposure to THF by inhalation

Exposure protocol		Control		600 mg/m^3		1,800 mg/m^3		5,400 mg/m^3	
5 Exposures									
BrdU labeling (% of control)		**%**	**LI[a] (%)**	**%**	**LI (%)**	**%**	**LI (%)**	**%**	**LI (%)**
	Zone 1	100	1.01	110	1.11	122	1.23	143	1.44
	Zone 2	100	2.54	98	2.48	117	2.96	183[b]	4.66
	Zone 3	100	0.85	147	1.25	188	1.60	401[b]	3.41
	Zone 1, 2, 3	100	1.46	110	1.61	132	1.93	217[b]	3.17
Hematoxylin and eosin: MI		**MI (%)**		**MI (%)**		**MI (%)**		**MI (%)**	
	Zone 1	0.01		0.01		0.03		0.04	
	Zone 2	0.14		0.14		0.17		0.48[b]	
	Zone 3	0.00		0.01		0.00		0.19[c]	
	Zone 1, 2, 3	0.05		0.05		0.07		0.23[b]	
5 Exposures + 3-week recovery									
BrdU labeling (% of control)		**%**	**LI (%)**	**%**	**LI (%)**	**%**	**LI (%)**	**%**	**LI (%)**
	Zone 1	100	0.88	120	1.06	100	0.88	109	0.96
	Zone 2	100	2.75	107	2.95	85	2.35	76	2.08
	Zone 3	100	1.09	170[b]	1.85	148	1.61	137	1.49
	Zone 1, 2, 3	100	1.57	124	1.95	103	1.61	96	1.51
Hematoxylin and eosin: MI		**MI (%)**		**MI (%)**		**MI (%)**		**MI (%)**	
	Zone 1	0.00		0.00		0.01		0.00	
	Zone 2	0.02		0.01		0.04		0.08	
	Zone 3	0.00		0.00		0.04		0.03	
	Zone 1, 2, 3	0.01		0.00		0.03		0.04	
20 Exposures									
BrdU labeling (% of control)		**%**	**LI (%)**	**%**	**LI (%)**	**%**	**LI (%)**	**%**	**LI (%)**
	Zone 1	100	1.39	106	1.48	91	1.27	104	1.45
	Zone 2	100	3.53	86	3.02	95	3.35	118	4.16
	Zone 3	100	1.52	133	2.02	134	2.04	230[b]	3.49
	Zone 1, 2, 3	100	2.51	101	2.17	103	2.22	141	3.03
Hematoxylin and eosin: MI		**MI (%)**		**MI (%)**		**MI (%)**		**MI (%)**	
	Zone 1	0.05		0.05		0.01		0.05	
	Zone 2	0.04		0.16		0.32[c]		0.24[b]	
	Zone 3	0.01		0.01		0.07		0.20[b]	
	Zone 1, 2, 3	0.03		0.07		0.13[c]		0.16[b]	

[a]LI = labeling index.
[b]$p \leq 0.01$.
[c]$p \leq 0.05$.

Source: Adapted from BASF (2001a)

In addition, BASF (2001a) also evaluated BrdU labeling in the uterine epithelium of female B6C3F$_1$ mice. The study authors reported no statistically significant changes in this measure were detected for any of the treatment groups. However, the BrdU labeling index in the controls was high. In addition, the mitotic index in the uterine epithelium was not significantly affected by THF exposure, while the percent increase in mitotic index was increased for mice exposed to the highest concentration for 5 days followed by a 21-day recovery. The authors (BASF, 2001a) suggested that an unusually low number of mitotic cells identified in the control animals contributed to the apparent increase in mitosis. The number of apoptotic cells was increased (168% of controls) in the high-concentration group given a 21-day recovery period. However, the overall data do not suggest that apoptosis plays a major role in cell regulation by THF, since the corresponding concentration in groups exposed 5 or 20 days had no increase in apoptosis (TUNEL staining). In addition, the total number of stained cells was small, suggesting that THF does not induce a robust apoptotic response in the uterus.

CYP450 activity was also evaluated as part of this study to examine the potential role of metabolism in the mode of action for THF-induced liver tumors (Gamer et al., 2002; BASF, 2001a). Female B6C3F$_1$ mice were exposed nose only to average THF concentrations of 0, 598, 1,811, or 5,382 mg/m^3 (0, 199, 604, or 1,794 ppm), corresponding to the concentrations used in the NTP (1998) cancer bioassay. Five consecutive days of exposure to THF at the high concentration generated a statistically significant increase in CYP450 content in the liver (125% of controls; $p \leq 0.05$), EROD activity (192% of controls; $p \leq 0.01$), and PROD activity (321% of controls; $p \leq 0.05$). EROD activity is often used as a measure of CYP1A family activity, while PROD is often used as a measure of CYP2B family activity, although there is some overlap in the specificity of these assays for various CYP450 isoforms among species (Weaver et al., 1994). This result would suggest that THF might be metabolized by CYP1A/2B isoforms, although these data do not provide direct evidence of their involvement.

In a second study by BASF (van Ravenzwaay et al., 2003; BASF, 2001b) female B6C3F$_1$ mice were exposed to THF concentrations of 0, 5,512, or 14,739 mg/m^3 6 hours/day for 5 consecutive days. The target concentrations of 5,400 and 15,000 mg/m^3 were chosen to match the high-concentration groups in the subchronic NTP (1998) study. Two groups of mice were used for each THF concentration. One group of mice was pretreated (about 1 hour prior to each exposure) with an i.p. dose of 100 mg/kg 1-aminobenzotriazole (ABT), a potent inhibitor of

CYP450 enzyme activity that has broad activity for many CYP450 isoforms. The parallel exposure group did not receive this pretreatment with ABT and was used to test the effects of THF without CYP450 inhibition. The livers of the mice were evaluated for total CYP450 content and some of the CYP450 activities including EROD, PROD, and nitrophenol hydroxylase (NPH), as well as cell proliferation (as measured by PCNA staining), and examination by electron microscopy.

Exposure of animals at the high concentration induced a narcotic effect. Three of 18 mice died in the high-concentration group without CYP450 inhibition, and 1 of 18 mice died in the high-concentration group pretreated with ABT. The high-concentration mice also had reduced body weight compared with controls. No clinical effects of THF were observed at the low concentration. No THF-related histopathology changes were observed in any of the treatment groups, although, in the livers of ABT-pretreated mice, centrilobular fatty changes were noted. Measurements of CYP450 content and activity revealed that CYP450s were induced in the high-concentration mice. Liver CYP450 content was increased by 98% in the high-concentration group, and this increase was blocked by ABT pretreatment. THF treatment induced PROD activity by about sixfold in the high-concentration group. In the mice pretreated with ABT, PROD activity was induced by approximately twofold by THF. EROD activity was increased by 160% in the high-concentration mice as compared to controls in the absence of ABT, and no induction of EROD activity was observed in the mice pretreated with ABT. These results show that THF induces both EROD and PROD activity and that the ABT pretreatment was an effective inhibitor of CYP450 isoform activity. In contrast to the results for PROD and EROD, NPH activity, known to be predominantly catalyzed by human and rat CYP2E1 (Kobayashi et al., 2002; Tassaneeyakul et al., 1993) was decreased in a concentration-dependent manner by THF and was not affected by ABT pretreatment. CYP450 content or associated enzyme activities were not induced above basal levels in the low-concentration group.

THF exposure induced cell proliferation at the high concentration, regardless of pretreatment with ABT. In mice exposed to 14,739 mg/m^3 THF without ABT pretreatment, PCNA staining was increased 814% relative to controls in zone 3, although a decrease to 59% of control levels that was not statistically significant was observed in zone 2, and no difference was observed for zone 1. The overall increase in PCNA staining for the three zones (pooled data) was 133% of controls (not statistically significant). In the high-concentration group pretreated with ABT, cell proliferation was even greater than the parallel THF group without pretreatment. PCNA staining was 150, 280, and 1,050% of control levels in liver zones 1, 2, and 3, respectively. In ABT-pretreated mice, the overall PCNA labeling for the three zones (data pooled) was 329% of controls. No change in PCNA staining was observed in the low-concentration groups regardless of pretreatment with inhibitor.

The data indicated that THF is an inducer of CYP450s and that THF induces cell proliferation in the livers of female mice, particularly in zone 3 hepatocytes. Pretreatment with the CYP450 inhibitor ABT enhanced the degree of PCNA staining, suggesting that THF itself, rather than a downstream oxidative metabolite, is responsible for the cell proliferative response. In mice with enzyme inhibition, the cell proliferation response was enhanced only moderately. It is possible that this effect would have been even more dramatic if the basal as well as inducible CYP450 activity had been blocked by the ABT pretreatments. ABT did not provide a complete inhibition of response, producing some uncertainty about the role that CYP450s play in THF-induced cell proliferation. A second area of uncertainty is that there were qualitative differences in the histopathology in the ABT-pretreated mice (i.e., centrilobular fatty changes) compared to mice without ABT pretreatment. It is not clear whether these histopathological changes that were unique to ABT-pretreated mice could have caused hepatocytes to be more susceptible to THF-induced liver toxicity. Even though these areas of uncertainty remain, the most possible interpretation of the data is that the cell proliferative response of the liver in female mice is not dependent on CYP450 activity, since treatment with the CYP450 inhibitor did not decrease the proliferative response. This interpretation suggests that THF itself, not a metabolite, is the active moiety in inducing cell proliferation. However, in the absence of further in vitro (or in vivo) metabolism data with and without ABT, it is not possible to determine if THF metabolism is actually inhibited and to what extent.

C.2.2.3. *Initiation*

Other than the NTP (1998) study, no direct animal cancer bioassays have been conducted. The use of THF as a solvent control in cancer studies for other compounds provides some limited data on the potential cancer mode of action for THF. Sawyer et al. (1988) evaluated the tumor-initiating properties of dibenz[a,j]anthracene, cholanthrene, and their diol and epoxide metabolites on the skin of SENCAR mice. The test compounds were dissolved in either acetone (30 mice/group) or THF (24 mice/group). The number of papillomas/mouse and percent of mice with papillomas was lower for THF-treated controls (5%) than for acetone-treated controls (16%) and was much lower than for the animals treated with the test compounds (39–97% for various treatment groups), suggesting that THF is not a potent tumor initiator. However, interpretation of this study is limited for a number of reasons. The study authors did not provide data on the historical incidence of papillomas. A tumor-screening protocol was used, which did not include a control group, an adequate number of dose levels, or adequate numbers of animals/dose group. Another complication in evaluating this study is that the tumor incidences for the test compounds dissolved in acetone or THF could reflect cocarcinogenic interactions.

C.2.2.4. *Inhibition of Gap Junctional Intercellular Communication*

Chen et al. (1984) investigated the ability of organic solvents to inhibit gap junctional intercellular communication (GJIC). Cocultures of 6-thioguanine-sensitive and resistant Chinese hamster V79 fibroblast cells were treated with the test compound and the degree of metabolic cooperation was determined by the survival of the resistant cells. The killing of resistant cells serves as an indicator of metabolic cooperation, because the toxic 6-thioguanine metabolite that is formed only in the sensitive cells can be passed on to normally resistant cells when gap junctions are intact. Therefore, robust growth of the resistant cells in this assay system would suggest that GJIC is inhibited. THF was judged to be positive (as defined by a doubling in recovery of resistant colonies) in the metabolic cooperation assays, suggesting that THF can inhibit GJIC. The recovery rate of resistant cells increased with increasing concentration (up to 100 μL of THF/5 mL of medium).

C.2.2.5. *Genotoxicity Studies*

THF was evaluated in a series of in vitro and in vivo assays to assess its potential for mutagenicity and genotoxicity and the results are summarized in Table C-5.

Table C-5. Summary of studies on the direct mutagenicity/genotoxicity of THF

Endpoint	Assay system	Results (without/ with activation)	Comments	Reference
In vitro studies				
Gene mutation —bacteria	*S. typhimurium* TA1535, TA1537, TA98, TA100	–/–	Used preincubation modification of the standard assay (NTP [1998] study)	Mortelmans et al. (1986)
	S. typhimurium G46, TA1535, TA100, C3076, TA1537, D3052, TA1538, TA98, *Escherichia coli* WP2, WP2 *uvrA*⁻	–/–	Gradient technique was used in which the mutagenic concentration range was identified as the lowest and highest concentration at which distinct colonies were observed; results presented in a summary table without data.	McMahon et al. (1979)
	S. typhimurium TA1535, TA1537, TA98, TA100	–/–	Screening only using a spot test was done in strains TA1535, TA1537, TA98; results presented in a summary table without data	Florin et al. (1980)
	S. typhimurium TA98	nt[a]/–	Results presented in summary text without data	Arimoto et al. (1982)

Table C-5. Summary of studies on the direct mutagenicity/genotoxicity of THF

Endpoint	Assay system	Results (without/ with activation)	Comments	Reference
Clastogenicity	Micronuclei, Syrian hamster embryo cells	nt/–	None	Gibson et al. (1997)
Chromosome aberration	Chinese hamster ovary cells	–/±	Slight increase with S9 not considered positive by study authors.; NTP study (1998)	Galloway et al. (1987)
DNA damage	Sister chromatid exchange, Chinese hamster ovary cells	–/–	Reported in the NTP (1998) study	Galloway et al. (1987)
Cell transformation	BALB/c-3T3 cells	–/nt	Limited activity was noted in one of two trials in the data tables, but not in the text of the study	Matthews et al. (1993)
	Syrian hamster embryo cells	–/nt	No cytotoxicity was observed at the highest test concentration	Kerckaert et al. (1996)
	NIH/3T3 cells	–/nt	THF used as control; cells treated in vitro were injected in mice to assess tumorigenicity	Collins et al. (1982)
In vivo studies				
Gene mutation	Drosophila sex-linked recessive lethal	–	Reported in the NTP (1998) study	Valencia et al. (1985)
Clastogenicity	Mouse erythrocyte micronucleus	±	Positive response only in mid-concentration males	NTP (1998)
Chromosome aberration	Mouse bone marrow	–		NTP (1998)
DNA damage	Mouse bone marrow, sister chromatid exchange	–		NTP (1998)
	Mouse hepatocyte unscheduled DNA synthesis	–	Reported in the NTP (1998) study	Mirsalis et al. (1983)

[a]nt = not tested.

Mortelmans et al. (1986) reported that THF did not induce reverse mutations with or without metabolic activation in four tester strains of the *S. typhimurium* test system. THF was also negative (with or without activation) when tested in a battery of eight strains of *S. typhimurium* and two *Escherichia coli* strains by using a modification of the standard assay (McMahon et al., 1979) or in four *S. typhimurium* strains (Florin et al., 1980). Several studies used or specifically examined the effects of THF as a soluble solvent in the *S. typhimurium* mutagenicity assays and generally support the conclusions of the above-mentioned more definitive studies. Hageman et al. (1988), in a study of the mutagenicity of frying oils, reported that THF solvent controls were nonmutagenic (with or without activation) in tester strains TA97, TA100, and TA104 relative to mutagen-containing oil samples. Maron et al. (1981) screened a series of solvents for compatibility with the *S. typhimurium* test system and reported that, while high-dose THF was toxic to the four tester strains used, it did not affect the mutagenicity of benzo(a)pyrene at lower levels (50 μL/plate) in strain TA100 in the plate incorporation protocol. THF was judged to be an unsatisfactory solvent for the preincubation assay due to higher cytotoxicity observed in this protocol modification. Finally, THF was reported to enhance the mutagenicity of tryptophan pyrolysate mutagens in *S. typhimurium* preincubation assay when used as a solvent (Arimoto et al., 1982). No potential mode of action for this effect was given, but the authors reported (no quantitative data provided) that the solvent was not itself mutagenic in tester strain TA98 with activation. The studies by Hageman et al. (1988), Arimoto et al. (1982), and Maron et al. (1981) are of limited value for assessing the mutagenic potential of THF because THF served as the control solvent in these studies and it is not clear if the results for THF were compared to untreated samples.

THF was also negative in a variety of in vitro assays evaluating chromosome and DNA damage up to cytotoxic concentrations. Gibson et al. (1997) reported that THF did not increase micronuclei formation when assayed in Syrian hamster embryo cells at concentrations that significantly reduced cell number. Galloway et al. (1987) reported some increase in total chromosome aberrations in the presence of S9 activation in Chinese hamster ovary cells. A majority of the aberrations were classified as simple, including breaks and terminal deletions. The study authors suggested that these increases were insufficient to be scored as a positive result. As part of this same study, Galloway et al. (1987) reported that THF did not induce sister chromatid exchanges in this cell system at cytotoxic doses.

THF was judged to be inactive when tested in the standard BALB/c-3T3 mouse cell transformation assay (Matthews et al., 1993). A Syrian hamster embryo cell assay was also negative for cell transformation when THF was tested at concentrations up to 5 mg/mL (Kerckaert et al., 1996). Collins et al. (1982) evaluated the in vivo tumorigenicity of NIH/3T3 cells transformed in vitro by benzo[a]pyrene-trans-7,8-dihydrodiol-9,10-epoxide (BPE) dissolved in THF. The ability of BPE-treated cells to induce tumors in normal mice (strain not specified) and AT×FL mice having a compromised immune response (thymectomized, lethally irradiated, and restored with syngeneic liver cells) was greater than the tumorigenicity of cells treated with THF only. Cells from 46/57 BPE-treated plates were tumorigenic in vivo, whereas cells from only 2/20 of the THF-treated plates were tumorigenic when injected in mice. The background tumor rate for untreated mice was not reported, but the low incidence of tumors induced by THF-treated cells as compared with positive controls suggested that THF did not significantly increase the rate of cell transformation.

THF has also generated negative findings in in vivo genotoxicity assays. THF did not induce sex-linked recessive lethal mutation in *Drosophila melanogaster* in a screening test for 48 chemicals for NTP (Valencia et al., 1985). NTP (1998) evaluated the formation of micronuclei in peripheral blood erythrocytes in male and female mice at the end of their 13-week inhalation study. There was only a statistically significant increased incidence of micronucleated normochromatic erythrocytes at the mid concentration in males. The effect was not concentration dependent, and no corresponding increase was seen for females. The results were considered by NTP to be equivocal. In a bioassay for chromosomal aberrations, male $B6C3F_1$ mice received THF by i.p. injection at doses of up to 2,000 mg/kg. No significant increase in the number of aberrations/cell or percent of bone marrow cells with aberrations was observed (NTP, 1998).

In vivo assays for DNA damage have also been conducted for THF. Male $B6C3F_1$ mice received THF doses of up to 2,000 mg/kg by i.p. injection. Bone marrow cells were harvested after 23 or 42 hours of exposure. In the 23-hour treatment protocol, a significant increase in the mean number of sister chromatid exchanges/cell was reported for the high-dose animals. However, this effect was observed in only one of the two replicate trials. No increase in sister chromatid exchanges was reported for the animals exposed for 42 hours. NTP (1998) characterized these results as negative. In another assay for DNA damage, Mirsalis et al. (1983 [published abstract]) reported that in vivo treatment of male rats with THF did not induce unscheduled DNA synthesis in hepatocytes.

Loureiro et al. (2004, 2000) reported formation of three DNA adducts from reaction of 2′-deoxyguanosine with trans, trans-2,4-decadienal occurring in the presence of oxidized THF. Later on, the same investigators structurally characterized these novel stable adducts produced by the reaction of THF oxidation products with 1,N^2-etheno-2′-deoxy-guanosine (Hermida et al., 2006; Loureiro et al., 2005). They also claim that an interaction leading to DNA-THF adducts may be a contributing factor to the observed toxicological effects associated with THF exposure. However, the limited information available from in vitro and in vivo genotoxicity studies point to THF as non-mutagenic (NTP, 1998). Further investigations are necessary to evaluate the possible interaction of THF oxidation products with DNA and their role in mutagenic mode(s) of action or THF-induced carcinogenic activity in rodents.

In summary, the genotoxic potential of THF has been evaluated in a variety of in vitro and in vivo assays. Nearly all the results are conclusively negative, with equivocal findings reported in a small number of assays that have been conducted. Taken together, these data support the conclusion that THF is not likely genotoxic.

C.2.3. Noncancer Mode of Action Information

THF was evaluated in a series of short-term in vitro tests to assess its potential for cytotoxicity (Matthews et al., 1993; Dierickx, 1989; Curvall et al., 1984; Pettersson et al., 1982). The results of these studies suggest that THF is not cytotoxic.

The available data suggest that THF metabolism is extensive and that oxidative metabolism may be due to CYP450 isozymes. However, the identity of the isozymes responsible for THF metabolism has not been elucidated. In addition, whether THF or one of its metabolites is responsible for the observed toxicological effects is unknown. Some mode of action data (BASF, 2001b) suggest that the parent compound might be the active form for liver toxicity and that metabolites might be responsible for neurological effects.

In the two-generation reproduction study (Hellwig et al., 2002; BASF, 1996) of THF in rats by the oral route, increased kidney weights in F0/F1 adults were observed in the high-dose groups. The mode of action for THF-induced kidney toxicity is unknown. Two possible modes of action were considered. First, THF exposure by the inhalation route induces CYP450 activity in the mouse liver (Gamer et al., 2002; BASF, 2001a, b), and therefore it is possible that a similar response could occur in the rat kidney. However, data available showed that acute inhalation exposures had no effect on kidney CYP450 activity in male F344 rats (Gamer et al., 2002; BASF, 2001a). These results are not directly comparable to the oral two-generation study since the exposure duration and rat strains differed between the two studies. Nevertheless, the only directly available data do not support the idea that CYP450 induction is responsible for the observed increase in kidney weight. Furthermore, since it is not known whether THF itself or a metabolite is the active moiety with respect to the kidney effects, it is not clear whether an induction of CYP450 activity is likely to increase or decrease THF toxicity in the affected organ. Some data suggest that an α_{2u}-globulin-associated mode of action could contribute to THF-induced nephrotoxicity. However, there is insufficient evidence to conclude that the kidney effects observed following THF exposure are related to the accumulation of α_{2u}-globulin for the following reasons (See Section 4.7.3.1 for analysis of the available data).

Decreased body weight gain in F1/F2 pups and delayed developmental stages (delayed eye opening) in F2 pups were also observed in the high-concentration groups of the two-generation reproduction study of THF in rats by the oral route (Hellwig et al., 2002; BASF, 1996). Two hypotheses for the observed decrease in pup body weight gain were considered. First, decreased maternal water intake during the lactation period could limit maternal milk production, resulting in decreased nutrition for pups and corresponding decreases in their growth, assuming that the composition of the milk did not change to maintain its nutritional value at times when water intake is low. Published studies have showed an association between water restriction and decreased volume of milk production in both humans and livestock (Hossaini-

Hilali et al., 1994; Morse et al., 1992; Dusdiecker et al., 1985; Little et al., 1980), and, therefore, the proposed explanation of decreased pup body weight gain due to decreased milk production is biologically possible. The temporal pattern of decreased pup body weight gain (significant decrements only during PNDs 4–14) correlates well to the postnatal lactation period where milk intake is greatest, and thus demand on a limited maternal milk supply would be expected to be most dramatic. The absence of an effect on pup body weight gain for PNDs 14–21 corresponds to the period where pups begin direct food and water intake and therefore depend less on milk production as a source of nutrition. Whether the observed decrease in water intake was due to a toxic effect of THF or was secondary to poor palatability is not clear from the available data. No study was conducted to test specifically whether THF, at the concentrations tested, reduced water intake solely because of taste aversion. Also, the two-generation study (Hellwig et al., 2002; BASF, 1996) did not include a water-restricted control group to separate the effects of decreased water intake from those that are induced directly by THF. In some cases the temporal pattern of water intake can provide evidence for decreased palatability, where decreased water consumption at initial introduction of the treated water is greater than the decrease observed at later exposure periods. However, for the two-generation study (Hellwig et al., 2002; BASF, 1996), the decrease in water intake was not greater for week 1 versus other weeks during the premating period. This result by itself is not sufficient to determine whether decreased water intake was secondary to palatability, since water intake data for initial days of exposure were not reported (weekly summaries were provided in the report), and this is only an indirect measure of potential taste aversion.

The second hypothesis is that THF itself induces a direct effect on pup development. Several considerations provide indirect support for a role of THF in the observed decreased pup body weight gain. In the two-generation study (Hellwig et al., 2002; BASF, 1996), THF induced developmental effects in the F2 pups (delayed eye opening and increased incidence of sloped incisors) in addition to decreased pup body weight gain. While this observation that other developmental indices are affected by THF treatment supports a role of THF exposure, it could simply reflect additional developmental delays resulting from decreased milk availability. The developmental effects of THF have also been tested in inhalation exposures in rodents, which would not be subject to issues of water palatability. However, the available studies did not assess postnatal development (sacrifice was at the end of gestation) and therefore do not provide directly comparable responses to the oral two-generation study. In the inhalation studies, maternally toxic concentrations of THF reduced fetal survival and weight and increased the incidence of fetal skeletal alterations in rats and mice (Mast et al., 1992; DuPont Haskell Laboratory, 1980). These inhalation data are consistent with the hypothesis that THF can induce developmental effects. On the other hand, even though the two-generation study did not fully evaluate fetal toxicity outcomes, the absence of a THF effect on litter size or pup body weight during the early postnatal period (days 1–4) suggests that fetal effects were not occurring in the oral dosing study. One explanation for the absence of an indication of fetal effects in the two-generation study, other than dose route, is that the degree of maternal toxicity in the inhalation studies was more severe than in the drinking water study. However, a subtle effect on male rat fertility/fecundity may exist following exposure to a high concentration of THF in drinking water based on a slight decrease (not statistically significant) in the mean number of delivered F2 pups and a finding of one infertile F1 parental male rat in the high dose group (Section 4.3.1).